Vol. XX. *May, 1911.* *No. 6*

BIOLOGICAL BULLETIN

EXPERIMENTAL CONTROL OF MORPHOGENESIS IN THE REGULATION OF PLANARIA.

C. M. CHILD.

The present paper is a brief account of certain results of experimental work upon *Planaria* which has extended with interruptions over the last ten years. The data presented here concern *Planaria dorotocephala* Woodworth, but *P. maculata* is similar in most respects. A complete account of the work will appear elsewhere.

1. The Dominance of the Anterior Region in Regulation.

My experiments support very positively the conclusion that in *P. dorotocephala* the anterior region, and more specificially the head region, controls the process of regulation in regions posterior to it and within a certain distance, *i. e.*, it is physiologically dominant over these regions. A limit of effectiveness for this dominance exists and this varies in general with the rate or intensity of the processes in the anterior region. Moreover, not only is the head region dominant over the regions posterior to it and within the limit of effectiveness, but in general a given level of the body is dominant over regions posterior to it within the limit and is dominated by regions anterior to it.

Before considering other facts it is necessary to recall that in nature all asexual individuals of *P. dorocephala* above a certain size consist of at least two zooids and the larger animals of more than two. This is shown by the following facts: (1) If the animals are cut into a series of small pieces of equal length from the anterior end posteriorly, the capacity for head formation decreases and disappears in successive pieces from the anterior end to about the middle of the postpharyngeal region, *i. e.*, the level where fission occurs, and then increases suddenly. This sudden

increase represents the anterior region of the second zooid. Where the second zooid is of considerable length a similar decrease in the capacity for head formation occurs from its anterior end to about the posterior one half to one third of its length, where again a sudden increase in the ability of the pieces to form heads occurs. Posterior to this level there is a more or less diffuse region of very great capacity for head formation probably in reality a series of head regions, extending to the end of the body. Other features of regulation, the rate, the relation between regeneration and redifferentiation, the size of the head and the position of the pharynx show corresponding regional changes. Most of these facts were briefly stated in an earlier paper (Child, '06*b*) and will be considered more fully elsewhere.

Secondly, it is possible by various means to induce fission in a very short time in animals which are far below the size at which fission would occur in nature, or which for other reasons would be incapable of fission under natural conditions. In all cases where fission can be induced it occurs at a level where sudden increase in the capacity for head formation appears. Some of the methods of inducing fission have been described in another paper (Child, '10*b*).

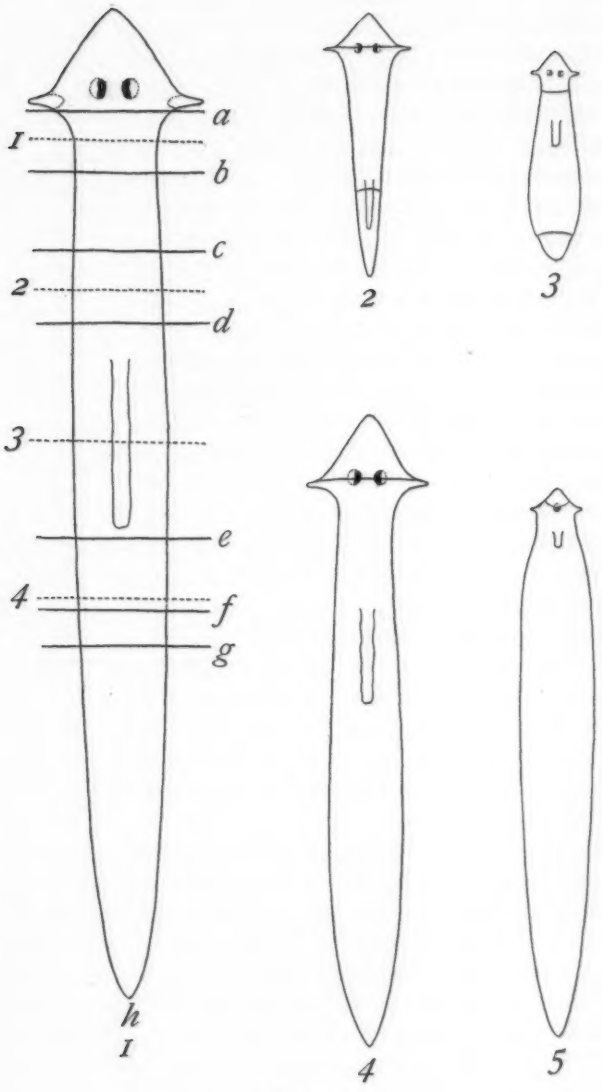
And finally, the length of the second zooid, together with other zooids which may arise from its posterior region varies with the length of the whole, but not proportionally. In very small animals the second zooid cannot be found by any methods thus far employed, but as the animal grows longer, it appears at the posterior end as a short region of high capacity for head formation and as growth continues it increases in length more rapidly than other parts; consequently in very large animals the postpharyngeal region is often double or more than double the length of the prepharyngeal, while in very small animals the prepharyngeal region is the longer. In *Planaria* the second zooid does not develop a head as visibly differentiated region before its separation, as do the posterior zooids of the Microstomidæ and annelids. This is because the processes which make this region morphologically posterior are more firmly fixed in *Planaria* than in those forms. So long as it remains attached to more anterior regions, the development of the second zooid can proceed only

to a certain stage because existing conditions inhibit it. But, as I have pointed out, this development does proceed far enough to permit some degree of independent motor reaction in the second zooid, and it is this which brings about fission.

From the fact of the existence of the second zooid it follows that the region which represents physiologically the posterior end of the first zooid is somewhere near the middle of the postpharyngeal region, or in very large animals, in its anterior third. If we wish to compare anterior and posterior regions of the same zooid, this fact must be kept in mind. Turning now to the question of the dominance of the head region, the facts which indicate this dominance are briefly as follows: A piece above a certain size from any level of the body is capable of reproducing all parts which lie posterior to its level whether it produces a head or not, but it is incapable of giving rise to any of the parts which lie anterior to its level, unless some approach to head formation occurs first.

For example, a piece like *cd*, Fig. 1, is capable of producing a new pharynx and the characteristic postpharyngeal intestinal branches, even though it may fail to form a head, as is the case under certain conditions. On the other hand the piece *ef*, from the postpharyngeal region, *i. e.*, the posterior region of the first zooid, never produces even a pharynx unless some approach to head formation occurs first. If it remains headless as it usually does, it also remains without a pharynx or mouth and no prepharyngeal intestinal region with an axial intestine ever appears. Anterior regulation in such cases is limited practically to closure of the wound.

The dominance of the head region is also clearly shown by the relation between the rapidity of development and the size of the head on the one hand and the position of the pharynx on the other. In pieces from near the anterior end (*e. g.*, *ac* or 12, Fig. 1) the head is disproportionately large and develops very rapidly and the pharynx is situated near the posterior end of the piece (Fig. 3), while in pieces such as *eg*, Fig. 1, from the posterior part of the first zooid, the head is relatively small and forms slowly and the pharynx is anterior to the middle of the piece, Fig. 3. Moreover, it is possible to demonstrate experimentally in pieces



FIGS. 1–5.

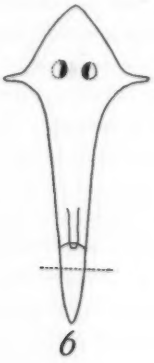
from the postpharyngeal region that any external conditions which alter quantitatively the processes which give rise to a head also alter the position of the pharynx. A single example is given here to illustrate this relation. The piece *eh*, Fig. 1, including the whole postpharyngeal region and the second zooid, always gives rise, under anything like natural conditions, to animals like Fig. 4 with large heads, normal eyes and the pharynx somewhat anterior to the middle. If, however, we decrease the rate of the metabolic processes by means of low temperature, anesthetics, CO_2, etc., the head forms much more slowly, remains much smaller, often possessses only a single median eye and the pharynx appears close behind the head and remains of small size. Fig. 5 shows the position of the pharynx in such a piece after regulation in 1.5 per cent. alcohol, 0.3 per cent. ether or in water containing CO_2. By varying the external conditions quantitatively all gradations between the conditions of Figs. 4 and 5 can be obtained. Changes in the other direction can be brought about by high temperature and also by other conditions which increase the rate of reactions in the body. In such cases the head is larger and develops more rapidly and the pharynx lies further posteriorly.

In general I have found it possible by these and other methods to alter the localization of the pharynx in pieces within very wide limits. Since the pharynx is situated at the posterior end of the median axial intestinal branch of the prepharyngeal region, the localization of the pharynx in the piece is directly related to the length of this axial intestine. In these experiments a definite spatial relation between the rate of the dynamic processes and the localization and size of organs and regions appears.

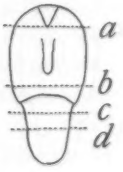
Moreover, the dominance of the head region and the limit of its effectiveness also appear in the conditions determining the origin of a second zooid. In newly hatched *P. maculata*—I have not as yet had opportunity to examine newly hatched *P. dorotocephala*, but have no doubt that they are similar—the second zooid is not present and even the whole postpharyngeal region is incapable of forming a head when isolated. In this respect it is like the posterior region of the first zooid in larger animals. As the worm grows longer the capacity to produce a head appears

posterior to a certain level, and once established, the second zooid grows nore rapidly than the first. When the animal is 5–8 mm. in length a very short second zooid is present and in animals sightly longer than this fission may be induced experimentally (Child, '10*b*). In the posterior region of the second zooid a third zooid arises early because the head region of the second zooid is not sufficiently active to control more than a short region; the extreme posterior region of the body often seems to consist of a series of very short head regions, reminding one of the series of minute segments which often appear in the growing posterior region of various annelids.

Under experimental conditions it is possible to delay or inhibit the appearance of the second zooid in regulating pieces or to accelerate it. For example, if we isolate the region anterior



6



7

FIGS. 6–7.

to the level *c* or 2 in Fig. 1, including the old head, it forms an animal more or less like Fig. 6, in which the head and prepharyngeal region are disproportionately large. If now after the growth and differentiation of the posterior new tissue is completed, we isolate the posterior end at the level indicated by the dotted line in Fig. 6, we find it does not produce a new head, *i. e.*, it is physiologically as well as morphologically a posterior end. If we feed pieces like Fig. 6 so that growth occurs, we shall find that after they attain a certain length the second zooid appears and after this the posterior region of the animal shows a high capacity for head

formation. But such animals must grow to a much greater length than young normal animals before the second zooid appears, because in them the dominance of the head region is effective over a greater distance than in young aninals, and a sufficient degree of physiological isolation (Child, '10*b*, '11*a*) of the posterior region does not occur until a greater length is attained

On the other hand, in pieces of *Planaria* which remain headless second, third and further zooids arise at once and if the pieces can be induced by stimulation to move about sufficiently, fission often occurs. The headless piece, unless very small, always produces a very large amount of new tissue at its posterior end (Fig. 7). This new tissue consists of new zooids; if we cut it off at any of the levels *b*, *c*, *d* (Fig. 7) we find that it always produces a normal whole very rapidly. If we remove the anterior end of the headless piece at the level *a* in Fig. 7 after the second zooid has formed at its posterior end we find that it is now capable of forming a normal head, but this is possible *only when the second zooid is present*, for if we remove both the anterior end and the second zooid, the remaining piece is incapable of forming a head. This last experiment as well as others to be described elsewhere shows that the second zooid influences regions anterior as well as those posterior to its anterior end.

In general any condition which retards or inhibits the development of the head or the dynamic processes in the developed head favors the development of a second zooid at the posterior end and any conditions which accelerate the dynamic processes in the anterior region retard the development of the second zooid. As noted above, the length which the animal attains before it gives rise to a second zooid may also be varied experimentally. In these experiments we have a demonstration of the spatial limit of effectiveness of physiological correlation and of the relation between this limit and the rate or intensity of dynamic processes in the region of origin.

One other point may be added as indicating the dominance of the head region. In animals which decrease in size in consequence of starvation the head region decreases less rapidly than other parts and so becomes relatively larger the further the reduction proceeds. Manifestly the head region is able to live

largely at the expense of other parts, to use their substance in maintaining its own structure.

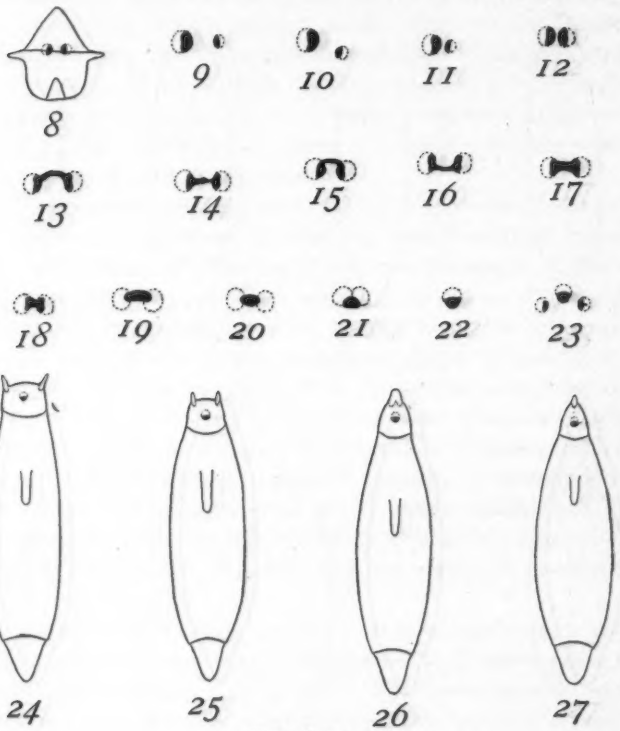
Only a few few brief suggestions as to the occurrence and general significance of dominant and subordinate regions in organisms are possible here. The dominance of the growing tip over other regions in the plant is a well established fact: in my work on *Tubularia* (Child, '07*a*, *b*, *c*, *d*) I called attention to various facts which indicate that the hydranth region is physiologically dominant over other parts and the same is true for *Corymorpha* and many other, perhaps all hydroids and for all actinians with which I have worked. Moreover, when we recall that in most if not all animals development of the egg begins at the animal pole and that the distal or anterior region arises at or near this pole, the possibility that the "animal" distal or anterior region is very generally dominant becomes at once apparent. I believe that we have here a very general law of development which has not been clearly recognized. It is probable, however, that in many forms the specification of different regions of the egg becomes fixed to such an extent that their further differentiation is to a greater or less extent constitutional rather than correlative, but there are various facts which indicate that within each such "self-differentiating" part we shall find a condition of dominance and subordination of parts more or less similar to that which exists in the whole egg and often even in the adult of some other less highly differentiated forms.

II. The Regulatory Development of the Anterior End.

The "normal" head of *P. dorotocephala* possesses the form shown in Fig. 1. As in various other species of *Planaria* the eyes consist of black pigment spots and unpigmented sensory areas. The sensory cephalic lobes or auricles are also only slightly pigmented and are marked off as well-defined light areas from the rest of the dorsal surface of the head, which is dark brown. The light areas of the eyes and the auricles are indicated in Fig. 1 by dotted lines.

The result of regulation in the larger isolated pieces is usually a "normal whole" (Fig. 4), *i. e.*, and individual with head essentially like Fig. 1, though varying in size, and the other organs

characteristic of the species in nature. In pieces below a certain size which varies with the region of the body and with other internal and external conditions, normal wholes develop from isolated pieces but rarely or not at all. A brief description of some of the characteristic results of regulation under these conditions



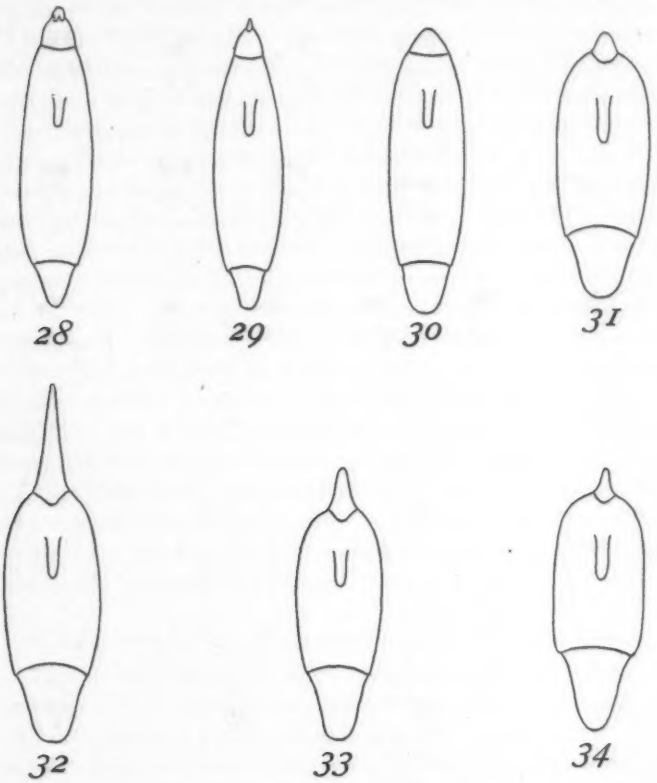
FIGS. 8–27.

is necessary. For convenience these are arranged under a number of heads, but it must be understood that the "types" thus distinguished are by no means sharply defined. They grade into each other in various ways, so that the whole series of types is actually a continuous series.

1. *Tailless.*—(Fig. 8.) Very short pieces from the more anterior regions often give rise, especially under certain experi-

mental conditions to heads without pharyngeal or postpharyngeal regions.

2. *Normal.*—(Figs. 2, 3, 4, 6.) In larger pieces these are all much alike (Fig. 4), but in smaller pieces they differ according to the region of the body and other internal and external factors.



FIGS. 29–34.

Short pieces from the anterior region produce normal wholes like Fig. 2, those above a certain size from the pharyngeal region or immediately posterior to it produce wholes like Fig. 3.

3. *Teratophthalmic.*—(Figs. 9–23.) Abnormalities of the eyes are frequent in regulation of pieces of *Planaria* and their occur-

rence has been noted by various authors, though no one has previously attempted to determine the conditions of their appearance. They are of various kinds, but in most cases fall into one of the following groups: differences in size or asymmetries of position (Figs. 9–11); approach to the median line (Figs. 11–12) partial union of pigment areas (Figs. 13–19); single median pigment areas with double sensory areas (Figs. 20–21); single median eyes (Fig. 22); three eyes, one median, others right and left (Fig. 23). In all of these cases the head may be entirely normal otherwise, though the more extreme types of abnormal eyes (*e. g.*, Figs. 20–22) usually occur in heads of relatively small size and slow rate of development.

4. *Teratomorphic.*—(Figs. 24–27.) Very frequently, however, the head itself is abnormal in form, the median anterior region being incompletely developed or absent and the auricles appearing on the anterior end of the head and in various degrees of approach to the median line: they may be separated by a considerable interval (Figs. 24, 25), or partially (Fig. 26) or completely united in the median line (Figs. 27). As soon as the new heads become pigmented the unpigmented auricular areas are clearly visible and in such cases as Figs. 26 and 27 it is these areas which enable us to identify with certainty the auricles. Teratomorphic heads almost invariably possess only a single median eye, or they develop a single median eye first and later two more symmetrically placed eyes (Fig. 23); they are always of small size and develop slowly.

5. *Anophthalmic.*—(Figs. 28–34.) These pieces show a distinct outgrowth of new tissue at the anterior end, varying widely in form, but always without eyes. This outgrowth is well innervated and its motor activities resemble those of a head. Sometimes the partially or wholly fused auricles appear anteriorly in the median line on the outgrowth (Figs. 28, 29), as in the extreme type of teratophthalmic pieces (Figs. 26, 27), but usually the outgrowth shows no visible differentiations characteristic of a head. In shape the outgrowth ranges from a broad and blunt form like Figs. 30 and 31 to a very slender form of varying length, often almost as long as the remainder of the piece (Figs. 32–34). These pieces always give rise to a new

pharynx whether they arise from the old prepharyngeal, pharyngeal or postpharyngeal regions.

The anophthalmic pieces are always more active than the headless pieces described below and their movements are better coordinated; moreover, the motor activity of the anterior outgrowth itself shows very clearly that it represents some approach to head formation.

FIGS. 35–37.

6. *Headless.*—(Figs. 35–37.) The headless pieces are distinguished from the anophthalmic by the fact that the new tissue merely fills the contracted wound and does not grow out. According to the degree of contraction of the wound these pieces may appear like Fig. 35 or Fig. 36. Headless pieces which arise from the old prepharyngeal or pharyngeal region always develop a new pharynx (Figs. 35, 36), but these from the postpharyngeal region do not give rise to a pharynx (Fig. 37).

It will be observed that in the order figured types 2–6 present a gradation from the normal head to the headless condition. The eyes first show irregularities, then appear nearer together and finally in partial or complete fusion in the median line. In the one eyed forms the region between the auricles may be reduced or absent and the auricles appear in various degrees of approach to each other, or like the eyes partially or wholly united in the median line, and this latter condition is sometimes seen in the anophthalmic forms: in these all possible gradations appear between an outgrowth resembling a head in form to a long slender pointed outgrowth or one which is short and small, and finally this condition passes into the completely headless condition.

The similarity of the first part of this series to the abnormal and cyclopian fish embryos obtained by Stockard is at once apparent.

III. Experimental Control of the Charater of the Anterior End.

Attempts to control the appearance of the various types of anterior end were first made in my experiments some five years ago and it was found that various methods of control were possible. The anesthetics were first used for this purpose almost three years ago; a brief report of certain phases of this work has already appeared (Child, '10*a*). Thus far I have been able to control the process of head formation through various internal and external factors.

In connection with these experiments it has become necessary to standardize the material as far as possible, *i. e.*, to obtain some method of comparing the physiological condition of worms of different size and age, of well fed and starved animals, of animals before and after regulation, pieces from different regions, etc. By the use of alcohol of low concentration (in most cases 1.5 per cent.) I have been able to accomplish this to a considerable extent. A part of the results of this work in their bearing upon the problem of senescence have been described elsewhere (Child, 11*b*). These experiments confirm the conclusion reached from experiments on regulation alone, viz., that in general the capacity for head formation stands in close relation to rate of the metabolic processes in the piece.

For given conditions a certain rate of reaction is necessary for the formation of a normal head. When the rate falls below this critical level teratophthalmic heads appear first, then as the rate falls still further the teratomorphic and anophthalmic and headless forms appear in succession. Moreover, among the teratophthalmic forms the cases of partial fusion represent decrease in rate below the critical level and the single median eyes further decrease. The irregularities of form and position occur most frequently at levels near the old head in small worms and in small pieces from large worms.

Since length of the piece, region of the body and physiological

condition are all factors in the capacity for head formation, pieces which are to be directly compared must be of the same size, except of course where the size factors are under consideration, they must be taken from the same region of the body except where the regional factor is to be analyzed and from individuals in similar physiological condition except where different physiological conditions are being compared. As noted above a relative measure of physiological condition is possible; it is also possible to control the size of the piece and the region of the body from which it comes within certain limits, especially in the pharyngeal region and near the head where localized morphological landmarks exist.

The method of experiment which I have found most satisfactory is to compare considerable numbers of similar pieces rather than individuals. For a temperature experiment for example, animals of the same size and as nearly as possible in the same physiological condition are taken as the basis: from these pieces as nearly as possible of the same size and from the same region of the body are cut and a number, usually fifty, of these pieces is placed in one temperature and a like number in another. For an alcohol experiment fifty pieces obtained in a similar manner are placed in alcohol and fifty in water at the same temperature and under otherwise similar conditions.

By taking into consideration the factors of size of piece and region of the body it is possible to cut series of pieces which will give 100 per cent. or very nearly of normal heads, or on the other hand 100 per cent. or very nearly of headless forms, or we can obtain series which in consequence of unavoidable differences in size and region and differences in the physiological condition of the animals from which they were taken, produce a certain percentage of several of the types described above. In all these cases we can compare the results under the control conditions with those occurring under other definitely known conditions.

For example, pieces above a certain size from the anterior region of the body, which give 100 per cent. or nearly of normal heads in well aerated water at a temperature of 20°C. show a large percentage of teratophthalmic forms in water at 10°C. or in

water containing CO_2 or in alcohol 1.5 per cent. at the same temperature as the control, and under more extreme conditions the teratomorphic, anophthalmic and headless forms appear.

On the other hand, if we take pieces which show nearly 100 per cent. of headless forms in well aerated water at 20° C. we find that similar pieces kept at 30° C. will produce not only anophthalmic, teratomorphic and teratophthalmic heads, but a considerable percentage of normal heads. These illustrations will serve to indicate the general character of the experiments along this line.

Below are given the results of a few series of experiments on size, region, temperature, alcohol, nutrition and mechanical stimulation.

1. *Size and Region.*

We may investigate these factors by cutting the whole body of worms of the same size and similar condition into one-fourth, one-sixth, one-eighth, one-twelfth and one-sixteenth pieces, etc., and comparing the results. The following table is a part of a series of this kind and shows in somewhat abbreviated form the results for one-eighth and one-sixteenth pieces of large worms. The head is not included and the pieces of the body are numbered consecutively 1, 2, 3, etc., from the anterior end backward; the consecutive numbers in the second column refer to the pieces. The remaining columns show the percentages of each of the regulatory types for each set of pieces. In this series each set consisted of only ten pieces, but the results are almost as uniform as in much larger series.

In this table the factors of size and region appear clearly. We see that the capacity for forming normal wholes decreases posteriorly and then in the region of the second zooid increases again suddenly. The anterior ends of the one-eighth pieces respectively are at approximately the same levels as those of nos. 1, 3, 5, 7, 9, 11, 13, 15 of the one-sixteenth pieces and it is at once evident that the longer one-eighth pieces possess a greater capacity for head formation than the shorter one-sixteenth pieces.

We can obtain a more striking expression of this fact by comparing very long and very short pieces from similar worms cut with anterior ends at the same level. One of my series of this

		Tailless Heads.	Normal Wholes.	Teratophthalmic and Teratomorphic.	Anophthalmic and Headless.	Dead.
One-eighth Pieces	1		100			
	2		10	90		
	3			20	80	
	4			10	90	
	5			10	90	
	6		10	20	70	
	7		80	20		
	8		90	10		
One-sixteenth Pieces	1	70	30			
	2		30	60	10	
	3		10	30	60	
	4		10		80	10
	5				100	
	6				100	
	7				100	
	8				100	
	9				80	20
	10			10	70	20
	11				70	30
	12			20	60	20
	13		20		60	20
	14		40		50	10
	15		80	10	10	
	16		90	10		

sort is as follows: from large worms of equal size and similar conditions fifty pieces were cut including the whole postpharyngeal region (*eh*, Fig. 1); a second set of fifty short pieces from the anterior end of the postpharyngeal region (*ef*, Fig. 1), *i. e.*, with anterior ends at the same level as the larger pieces, was used for comparison. The following table gives the results in percentages.

	Normal.	Teratophthalmic.	Teratomorphic.	Anophthalmic.	Headless.	Dead
Long	100	0	0	0	0	0
Short	0	0	2	10	82	6

In the same way we may compare other levels of the body and show to what extent the power of head formation depends upon the cells near the anterior cut end and to what extent upon their connection with more posterior regions. My experiments along this line show that in general the ability to form heads in pieces from the posterior region of the first zooid is almost wholly dependent upon their connection with more posterior regions, while in pieces from the anterior end of the body it is very largely independent of such connection.

2. *Nutrition.*

My experiments along this line are rather extensive and the results are of considerable interest. I give here only a single series of experiments. The pieces used included approximately the region between the lines 2 and 3 in Fig. 1; fifty pieces were cut from worms which had been fed every 2–4 days for two months (I), another set of fifty pieces from worms of approximately the same size which had had no food for forty days before the experiment began (II). Results are given in percentages.

	Normal.	Teratophthalmic.	Teratomorphic.	Abophthalmic.	Headless.	Dead.
I.	4	46	6	30	14	0
II.	0	0	2	10	64	24

The capacity for head formation is manifestly much greater in the well-fed (I) than in the starved pieces (II).

3. *Temperature.*

Under this head only a single series is given, but the results are characteristic. In this series fifty pieces from similar worms including the region between the lines 3 and 4 in Fig. 1 were allowed to regulate at each temperature. Results are given in percentages.

	Normal.	Teratophthalmic.	Teratomorphic.	Anophthalmic.	Headless.	Head.
28°–30°.	74	22	2	0	0	2
18°–20.°.	22	40	2	14	22	0

The death of one individual (2 per cent.) at high temperature was purely accidental. The percentages show the general character of the results. With greater differences of temperature greater differences in the results can be obtained.

4. *Alcohol.*

The experiments with alcohol and other anæsthetics present certain complicating factors which will be considered fully elsewhere, but the effect of alcohol upon the capacity for head formation and upon the character of the head formed is readily demonstrated.

In the series presented here fifty pieces from similar worms, including the region between lines 2 and 3 in Fig. 1 were placed

in alcohol 1.5 per cent. for 48 hours after section and then removed to water; fifty similar pieces were placed in water for control. Results are in percentages.

	Normal.	Teratoph-thalmic.	Terato-morphic.	Anoph-thalmic.	Headless.	Dead.
Alc. 48 hrs.....	14	16	8	18	38	6
Water	20	44	2	26	8	0

A much larger percentage of headless forms appears in alcohol than in water.

5. *Complications in the Effects of Depressing Agents and Conditions.*

In the present paper I wish merely to record the fact that under certain conditions and within certain limits depressing factors increase the head-forming capacity in pieces, instead of decreasing it. As I shall show elsewhere, this result can be accounted for without difficulty. It depends largely upon the fact that the rate of metabolism in the cells at the anterior end of the piece which react to the wound increases in the course of this reaction, while that of more posterior regions of the piece remains essentially the same. The depressing factor decreases the rate in both regions and with a certain degree of depression the more posterior region will become almost quiescent while the anterior region which loses its previous differentiation in consequence of the wound reaction will still be active. Under these conditions the dominance of the anterior over the posterior region will be increased and a larger percentage of heads may be formed in spite of the depressing effect, simply because the anterior region is more capable of living, growing and differentiating at the expense of the posterior region. Under these conditions then the depressing effect will appear to be a morphogenic stimulus.

6. *Mechanical Stimulation.*

As is well known, the shorter pieces of the planarian body, especially those from the more posterior regions of the first zooid, very commonly move about but little until regulation has attained a certain stage; after this stage "spontaneous" movements occur. Larger pieces show these movements at

earlier stages and in still larger pieces, *e. g.*, after removal of only the head region, they are never absent. In general the pieces which show more motor activity when left undisturbed in the dishes and which react more readily to slight stimuli are those which have the greater capacity for producing new heads, *i. e.*, for becoming wholes. From a number of pieces it is possible within a few hours after section to select with a considerable degree of certainty those which will form heads, or which will show the greater degree of head forming capacity, merely by observing the differences in motor activity and reaction to slight stimuli.

With these and various other facts in mind I carried out experiments to test the effect of mechanical stimulation to movement upon the formation of heads. The pieces were cut of such size and such distance from the old head that they showed little or no "spontaneous" movement during the first few days after section. Various methods of stimulation were employed: one of these was that of tilting the broad flat dish in which they were kept so that each piece was out of water for a few seconds. This usually serves to induce locomotion, but it does not continue long after the pieces are again in water. Sometimes the pieces were loosened from the glass by currents from a pipette or were turned upon their dorsal surfaces with needles, after which they usually righted themselves. But the most commonly used and most effective method was that of stroking or gently pushing the pieces with a small soft camel hair brush. Usually a few seconds of such stimulation was followed by relatively long sustained locomotion. The stimulation began a few moments after section of the pieces and was repeated at least every hour and often every half hour from about 8.30 A.M. till 6 P.M. and again at least twice between 8 and 11 P.M. each day until the heads in such pieces as produced heads were well developed and the eyes visible. Each time that the pieces of the one set were stimulated the water of the dish containing the control was gently disturbed and stirred in order to avoid as far as possible differences in aeration. Care being taken not to touch the control pieces directly, the movement of the water almost never induced movement of the pieces. The dishes were kept

under as nearly as possible identical conditions of light and temperature.

Two series of experiments are given here. In the first the pieces included the region between the lines 2 and 3, Fig. 1, fifty pieces for stimulation (I) and fifty for control (II) being taken from similar worms.

	Normal.	Teratoph-thalmic.	Terato-morphic.	Anoph-thalmic.	Headless.	Dead.
I	0	28	8	32	32	0
II	0	8	8	26	58	0

The second series consists of pieces including the region between the lines 3 and 4 in Fig. 1 and from the same worms as the first. These pieces, being posterior to those of the first series and of approximately the same size produce heads less frequently. As in the first series, fifty pieces were used in the stimulated set (I) and fifty in the control (II).

	Normal.	Teratoph-thalmic.	Terato-morphic.	Anoph-thalmic.	Headless.	Dead.
I	4	10	2	18	64	2
II	0	6	2	12	80	0

The two pieces which produced normal heads in the stimulated set of this series were undoubtedly pieces which included the anterior end of the second zooid: the presence of this region even at the posterior end of a piece increases greatly its head-forming capacity. In both series the effect of stimulation appears unmistakably in the increased capacity for head formation. Without doubt it is not the actual performance of the movement itself but the stimulation which is the important factor. Stimulation means increase in the rate or intensity of the dynamic processes in the piece and this appears in increased and altered formative capacity. I believe that these and other similar experiments to be described elsewhere establish and confirm against all objections my position that the process of regulation is essentially a dynamic, or as I have often called it, a "functional" process or complex of processes.

7. *The Morphogenic Effect of Quantitative Changes in Conditions in General.*

In the temperature, nutrition and stimulation series the experimental conditions differ quantitatively from those of the control.

For reasons which I shall state later it seems probable that the factor of length of the piece is essentially quantitative in its effect upon head formation, and the regional factor is certainly very largely quantitative. The effect of alcohol and other anæsthetics is also quantitative, at least in large part.

The results obtained in these experiments point to the possibility of controlling and analyzing morphogenesis in a great variety of ways and of obtaining some insight into the nature of morphogenic processes themselves. As regards the data presented, there are many facts which indicate that the rate of oxidations or of certain oxidation processes is the chief factor in the results obtained.

From the morphological point of view the most important point is that certain organs may alter their localization, their form and their relation to each other and may finally disappear as the rate of the processes concerned decreases and vice versa. It is evident that under given conditions a certain rate of certain dynamic processes is necessary for the production of certain morphological effects.

IV. The Effect of Change of Position of Parts in the Body.

If we cut small pieces from the posterior region of the first zooid such for example as *ef*, Fig. 1, they will give 100 per cent. or nearly of headless forms, If, however, we cut out a large piece, *eh* (Fig. 1) and allow a head to form at its anterior end and then after a week or ten days cut out small pieces just behind the head, we shall find that they now produce 100 per cent. or nearly of forms like Fig. 2, *i. e.*, normal wholes. These pieces represent approximately the region which when taken from the original animal gave rise to 100 per cent. or nearly of headless forms. Their capacity for forming heads has been widely altered by their changed position in the body and more specifically I believe by altered correlation with other parts. The cells of such a region are not directly concerned in the formation of a head, but the more anterior their position in the new individual, the greater their head forming capacity becomes.

In a similar manner we can decrease the head-forming capacity

of parts which were originally near the anterior end and possessed the capacities characteristic of that region. We can make almost any portion of the prepharyngeal region into a pharyngeal region with corresponding change in regulatory capacity. The anterior region of the second zooid, with its very high regulatory capacity may be changed into the postpharyngeal region of a first zooid with a great decrease of regulatory capacity. Changes of this kind may be made almost at will merely by so arranging the experiment that the part in question is brought into a certain position with regard to other parts. It not only acquires a different structure but its capacities are altered. These experiments show a wide range of possibilities of influencing the morphogenic capacities of parts indirectly through correlation. I believe the point is of some general interest. In general terms these experiments show that the regulatory capacity of a part of the body may be altered through correlation. As regards a specific case, the head, they show that a part which when isolated originally possessed little or no capacity to form a head may have this capacity greatly increased by closer association with a region where a head is forming, even though the part itself is not directly concerned in the formation of the head. Such facts as these, possess, I believe, a certain significance in connection with the problem of inheritance. In these cases we actually alter the hereditary capacities of the part in question by altering its correlation with other parts.

HULL ZOOLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
March, 1911.

BIBLIOGRAPHY.

Child, C. M.

'06a Contributions toward a Theory of Regulation, I. The Significance of the Different Methods of Regulation in Turbellaria. Arch. f. Entwickelungsmech., Bd. XX., H. 3, 1906.

'06b The Relation between Regulation and Fission in Planaria. Biol. Bull., Vol. XI., No. 3, 1906.

'07 An Analysis of Form Regulation in Tubularia, I., Stolon Formation and Polarity. Arch. f. Entwickelungsmech., Bd. XXIII., H. 3. IV., Regional and Polar Differences in the Time of Hydranth Formation as a Special Case of Regulation in a Complex System. Ibid., Bd. XXIV., H. 1. V., Regulation in Short Pieces. Ibid., H. 2. VI., The Significance of Certain Modifications of Regulation: Polarity and Form Regulation in General. Ibid., 1907.

'09 The Regulatory Change of Shape in Planaria dorotocephala. Biol. Bull., Vol. XVI., No. 6, 1909.

'10a Analysis of Form Regulation with the Aid of Anesthetics. Biol. Bull. Vol. XVII., No. 4, 1910.

'10b Physiological Isolation of Parts and Fission in Planaria. Arch. f. Entwickelungsmech., Bd. XXX. (Festband für Prof. Roux), II. Teil, 1910.

'11a Die physiologische Isolation von Teilen des Organismus. Vortr. u. Aufs. u. Entwickelungsmechanik, H. XI., 1911.

'11b A Study of Senescence and Rejuvenescence, Based on Experiments with Planaria dorotocephala. Arch. f. Entwickelungsmech., Bd. XXXI., H. 4, 1911.

THE BIOLOGY OF THE RED-BACKED SALAMANDER (PLETHODON CINEREUS ERYTHRONOTUS GREEN).

M. ETHEL COCHRAN.

DISTRIBUTION.

Baird says, "Species of the genus *Plethodon* are found all across the North American continent." Boulenger makes the range of the subfamily Plethodontinæ North America, with possibly one species in the valley of the Rio de le Plata. Of the subfamily Plethodontinæ, Gadow states that it "is almost entirely American (with one species, *Spelerpes fuscus*, in Europe)," while Holmes says that "the Plethodontinæ form a large group which is mainly confined to America." In the last edition of his "Manual," Jordan gives the range of the family as "chiefly North America."

The "red-backed" salamander (*Plethodon cinereus erythronotus* Green) belongs to the Plethodontinæ and according to Jordan is confined to the eastern United States. Gadow says, "*Plethodon erythronotus* extends into Canada," while Boulenger would have it range in the eastern United States and Canada. Cope claims the species *Plethodon cinereus*, including all varieties, has an extreme range, being "found throughout the United States, east of the Mississippi River. It appears to be more abundant in the Middle States; its northern range is to the middle of Maine, Ontario and Michigan." Kingsley says it is the "most abundant salamander in the eastern United States."

Throughout Worcester County, Mass., this little salamander has proved very abundant. It is not an uncommon thing to find twenty or more during a short afternoon's walk. Every little wood has its dainty, shy, inhabitants who so easily may be overlooked.

HABITAT.

The tiny creatures are not visible to the casual observer, for on bright days they are always concealed beneath stones or fallen logs. Holmes says they are in damp situations under rocks or

decaying logs, while Montgomery found them near West Chester, Pa., "at all seasons, never in streams or boggy places, but in woods and hillsides beneath wood and stones, a strictly terrestrial species," Kingsley, on the other hand, claims "*Plethodon cinereus* is found everywhere in woods, under bark, logs and stone, in comparatively dry places." Miss Whipple speaks of its "being found far from any water supply."

Overturning a stone discloses a red-back at home. (Photograph by Dr. Miller.)

During a year's study of the species, they have been found in both damp and dry places, within five feet of a pond's edge and on rather dry, high slopes. In the daytime, they are always concealed. In one case, near a spring, a large sawdust pile that contained several planks furnished an abode for several salamanders.

One damp, dark day, when the rain was falling gently, three medium-sized individuals were found on the surface of the ground near stones from beneath which they had evidently come. In the same locality, five were found beneath stones; two were so placed that it seemed as if I had frightened them in before

aware of their presence. This idea was strengthened by finding one of the two in an ant's nest, a thing not found before. Of several specimens kept in the laboratory, in vivaria, a few would venture out of their retreats on dark days.

Rarely, if ever, are the salamanders found in treeless places, but they seem to have equal preference for pine, birch or mixed woods. All slopes, if shaded and of not too sandy a nature, seem to provide suitable dwellings.

The natural crevices beneath stones are utilized by the red-backs. (Photographed by Miss Gulick.)

It seems that no holes are made by the salamanders themselves, but that they utilize whatever is at hand. Allen noted holes, but was not sure whether the salamanders made them or not. In a pile of stones or in a sawdust heap, there are many natural openings connecting in labrinthian fashion which are typical. In the vivaria kept in the laboratory, the pieces of moss were placed above a layer of sand and charcoal; a small dish of water was sunk to the level of the moss and the spaces between the pieces of moss, between the moss and sand, and between the "well" and sand were utilized by the red-backs as homes and highways.

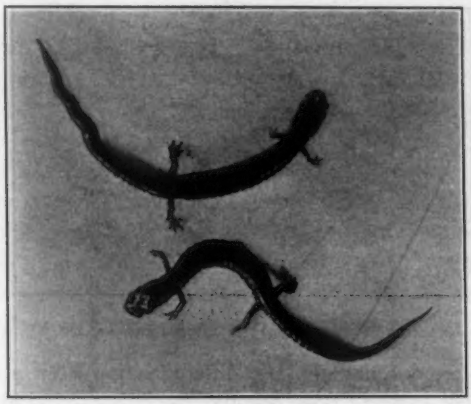
In one instance, where two specimens were found in rather soft earth, a well-defined hole was followed—it led by a short

gently-inclined path to a mass of rocks about a foot below ground where there were natural openings in all directions. The hole then appeared to be nothing but a natural opening worn smooth by use. Five other places were carefully studied where natural crevices seemed to be utilized.

The number of salamanders found under one object is variable; the most ever found was ten and countless times has only one been seen. There seems to be no evidence of their being or living in pairs during fall and early spring.

Description.

Kingsley calls these salamanders "the smallest in the United States." The largest specimen found measured 9.2 cm., while from 7.8 cm. seems to be the average length. The tail is about



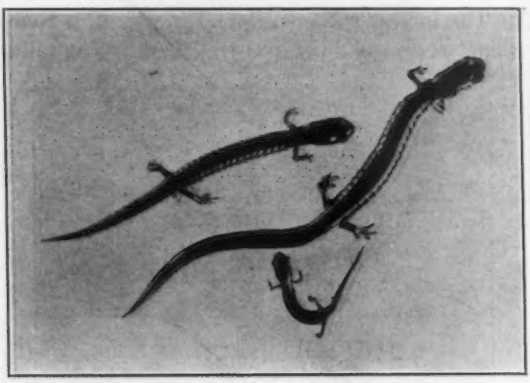
Red-backs. (Natural size.) (Photographed by the author.)

as long as the entire animal. Of two individuals, one, whose total length was 9.2 cm., had a tail 4.9 cm. long, while one 7.8 cm. long, had a tail 4.2 cm. in length. Jordan gives their total length as 3½ inches (9 cm.). The following measurements give some idea of the relative length and width of the body: total length, 8.4 cm., tail 3 cm., costals 2 cm., width of head 5 mm., width of body 4 mm., dorso-lateral diameter 2 mm.

They are slender, with an almost cylindric body and a sharply pointed cylindrical tail. The legs are thin and weak and the

feet proportionately small. The anterior foot has three toes and an inner rudimentary one, while the posterior has four with one inner rudimentary toe. The body is not always lifted from the ground when the creature is walking—the tail never. *Spelerpes bilineatus* seems to be more slender than *erythronotus*, and when a dusky salamander (*Desmognathus fusca*) is about, the daintiness of a red-back makes it seem as clumsily built as an *Amblystoma*.

The most distinguishing feature of this species is the broad dorsal band of color that varies from bright, brownish-red to a sort of pale, dead-leaf brown. Age seems to make no difference



Red-backs. (Natural size.) (Photographed by Dr. Miller.)

in the color, nor has any sex differentiation been observed in fall and early spring. Two peculiar specimens found in company with several normal ones, in widely separated spots, were of a bright vermilion red, similar to the color of young *Diemyctylus*, over all the dorsal surface; the tail alone was a dark brown. It was probably a chance variation, as it seemed normal in every other respect.

The sides of the body and much of the tail are of a dark gray-brown finely flecked with silver spots that grow more numerous toward the ventral side where the color is almost white, varied with a few darker spots. The ventral part of the tail and legs is much darker than the belly, while the dorsal surface of the

legs is similar to the sides. The eyes are large for the size of the head, dark, iridescent, and very prominent. The nostrils are visible; the head is quite flat, the nose rounded, not pointed.

The skin is always moist and some amount of water is needed in the environment, for a specimen kept in a dry pasteboard box for a few hours was found in a much shriveled condition, dead.

The throat is in constant vibration which at times is very rapid; various counts were taken from seventy to one hundred and eighty a minute. The costals or costal folds, from sixteen to nineteen, are very easily seen. There seems to be no external character during fall and early spring to distinguish the sexes; in a few specimens dissected, the males were larger than the females.

Activities.

The red-backed salamander is almost entirely nocturnal. Jordan and Kingsley mention this fact, while of *Autodax lugubris* Hallow, which belongs to a genus close kin to *Plethodon*, Ritter says it is entirely terrestrial and nocturnal.

This salamander is quick of movement. Often when first discovered, it is found in a graceful, curled position, and seldom moves unless touched. If annoyed, it glides rapidly, sometimes into crevices or holes and sometimes into the moss or leaves, where it lies quietly hidden. In this rapid gliding movement, the tail is lashed from side to side, as if aiding the fragile feet and legs.

It is a great climber—individuals in the laboratory climb up the sides of a glass vivarium with great ease, using the moist, adhesive abdomen, as well as the feet and legs. They have been seen to do this on rather dark days as well as in the evening.

Kingsley mentions finding them out of doors on a "spear of grass or coiled at the apex of a rachis of a fern at a distance of from twelve to eighteen inches above ground."

There is a peculiar jumping habit common to the species—if one is held in the hand, a foot or more above the moss floor of a vivarium, it seems to gather itself together into a coil, and using its tail as an aid, it springs lightly to the moss. The whole process is indescribably quick. Kingsley says it "leaps by sudden unbending of the coiled body like some caterpillars."

The red-back's activities in the water are interesting. Jordan says it "rarely or never enters water." Miss Whipple has described it well: "Although certain lungless species may be more or less aquatic, their activities, even in water, are terrestrial. Various species will at first, when in an aquarium, swim to the surface, then around and around the edge of the aquarium as if seeking a means of escape, but at the instant active swimming ceases, the body sinks clumsily and heavily to the bottom, where they remain until disturbed or until another effort is made to escape. Lungless forms show on the whole little power to adapt themselves to aquatic life. Most are terrestrial in habit, some, *Plethodon cinereus* and *Plethodon glutinosus*, being found far from any water supply.

"The nares close as soon as the animal is submerged in water and remain so as long as the animal is in water. In a few cases, there were attempts at a feeble bucco-pharyngeal respiration, but even then the external nares were closed and water was drawn in and expelled through the slightly opened mouth."

Salamanders in the laboratory seem able to endure extreme cold, for in a vivarium the water was found with a thick coating of ice, but the salamanders were apparently unaffected.

The latest date of finding a salamander out of doors in the fall of 1909 was November 13. The first specimens this spring (1910) were found on March 20, on a warm, southern slope free from snow. At this time there was still much ice and snow in the woods all about. In midwinter, Montgomery says the salamanders are found deeper in the ground.

Food and Feeding Habits.

Regarding the frogs, salamanders, etc., Dr. Hodge says in "Nature Study and Life," "with one or two exceptions . . . they are all valuable insect destroyers, each for its peculiar haunts; they should be generally protected and utilized as beneficent forces in nature."

The red-back, like other amphibians, prefers live, moving food. On several occasions, bits of meat placed in the vivarium remained untouched for days and became covered with mould while if a piece were moved before a salamander, it was taken

eagerly. Small wriggling earthworms are enjoyed. In one case, a small toad tadpole was offered to a salamander whose attention was so attracted at once by the struggles of the tadpole that it promptly ate it. A companion at the same time ate two tadpoles. Small insects are captured and eaten with avidity.

A moving object is noticed very quickly. The salamander will follow it fixedly for a moment, then, with gaze still upon it, creep slowly after until close at hand, when a rapid spring is made for it. Often it appears to have difficulty in swallowing food—the eyes close as several slow gulps are made and some time elapses before another portion is taken. No use of the hand in feeding has been noted, as is commonly seen in toads and frogs.

As a means of determining the kind of food taken in nature fifteen stomach-contents were examined. The salamanders came from several widely separated districts.

In general, specimens taken in the morning showed fuller stomachs than those taken in the afternoon.

The following specimens were found in the fifteen stomachs examined: Ants of several kinds, beetles of several kinds, bugs, caterpillars (cut-worm), centipedes, earthworms, fleas, flies of several kinds, maggots (rat-tail), midges, mites, mosquito wiggler, plant lice, plant remains, pseudo-scorpion, slugs (*Limax*), spiders, spring-tail (*Thysanura*), sow-bug, wasp-like insects.

Below is given a list of the contents of five stomachs that may be taken as typical.

A salamander captured May 17, 1910, at four P.M. in a sawdust heap near a spring, Auburn, Mass., contained: four snout beetles, one yellow ant, one small spider and ten mites.

A salamander found under a stone in an ants' nest, April 28, 1910, in the afternoon at Auburn, Mass., contained: one earthworm (2.75 cm. long), two mites, two small beetles, and three maggots that were probably young ants.

A salamdander found at Holden, Mass., at eight A.M., on April 27, 1910, contained: one earthworm (3.5 cm. long), one centipede, one black fly, one spider, one cutworm, one ichneumon maggot (probably from the cutworm), one pseudo scorpion, one sow-bug, and one unknown insect (which had eaten two small snails).

A salamander found on the afternoon of November, 11, 1909, at Gates Lane, Worcester, Mass., contained: three slugs (*Limax*, 1, 4, 5 mm. long respectively), one plant louse, one small worm, and plant remains (probably accidental).

A salamander captured in Norcross Woods, Worcester, Mass., at eight A. M., May 9, 1910, contained: one earthworm (2.2 cm. long), one brown spider (3 mm. long), two mites, one beetle, one spring-tail, one mosquito wiggler, one *Syrphus* fly, and plant remains.

Enemies.

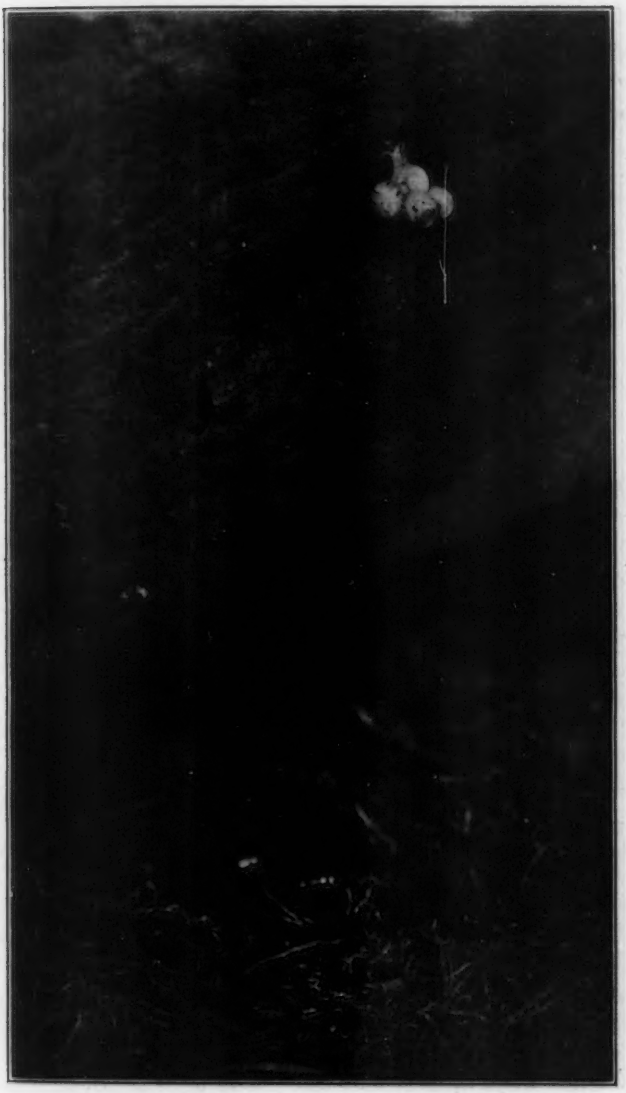
Snakes undoubtedly are great enemies of the salamanders. In a study made in Pennsylvania, Surface found that salamanders formed a large part of the diet of some snakes. The ribbon snake or striped garter snake (*Thamnophis saurita*), which belongs to the eastern United States and likes to live in shady, narrow, watered valleys, preys upon *beneficial* batrachians. "In four specimens containing food, four salamanders were found, two of which were *Plethodon cinereus;* 37.5 per cent. of the food of this species of snake was found to be salamanders." The common garter snake was found to destroy toads and salamanders among which was *Plethodon cinereus*. The water snake (*Natrix sipedon*) and the grass snake (*Liopeltis vernalis*) also were found to eat the red-back among other salamanders.

Ditmars states that the following snakes feed on salamanders, among which it is reasonable to include the red-back, as their haunts are much the same: ribbon snake (*Eutemia saurita* Linn.), mud snake (*Seminatrix pygœa*), brown or DeKay's snake (*Storeria dekayi* Hol.), green or grass snake (*Liopeltis vernalis*), eastern and western ringed-necked snakes (*Diadophis punctatus* Linn. and *D. amabilis* B. & G.).

A hungry, half-grown bull-frog ate a good-sized salamander with evident relish and a purple grackle treated one like a worm, beating it and breaking it with his bill before eating it.

Protective Devices.

Plethodon cinereus erythronotus, when annoyed, yields a colorless, glutinous secretion from its tail. A Californian salamander, *Plethodon oregonensis*, was found by Miss Hubbard to yield



An upturned stone, showing cluster of eggs, manner of attachment and brooding salamander. (Natural size.) (Photographed by Dr. Miller.)

on the tail an abundant poisonous fluid which did not prevent snakes from eating it. Easterly has written of the large granular glands of this salamander which are poisonous, but to what he does not state.

Baird speaks of the genus *Plethodon* whose skin exudes a highly glutinous secretion, but he makes no statement of its nature. Gadow says: "Numerous experiments have shown that the poison of toads, salamanders and newts is capable when injected, of killing mammals, birds, reptiles and even fishes, provided of course, the dose be proportionate to the size of the animal. Small birds and lizards succumb as a rule in a few minutes; guinea-pig, rabbits and dogs in less than an hour. This poison of amphibia is not septic, but acts upon the heart

Raised stone showing pair of red-backs with eggs. (About one half natural size.) (Photographed by Dr. Miller.)

and central nervous system. Some authorities hold that the poison is an acid, others regard it as an alkaloid."

The fluid from a red-back's tail is very sticky when placed in the mouth; after a short time, a slight biting sensation is felt for a few moments.

Several of the salamanders taken from their native haunts had every mark of possessing a regenerating tail. In one case, the tail had been cut or broken off nearly to the hind legs and the bud of a new one had grown out about a quarter of an inch.

Miss Towle found that *Plethodon cinereus* had the regenerative power also in the limbs.

Of *Plethodon oregonensis*, Miss Hubbard says it practices "autotomy only as a last desperate resource, and but in one region (directly behind the anus)." But when in two or three instances, the tail of a red-back has been held by forceps, the tail has come off very easily and in no particular region. Those found wild with incomplete tails appeared to have been broken in no particular joint. This salamander has not been observed thus far to snap or bite for protection.

Intelligence.

The red-backs are easily tamed, and will learn to eat food when offered on a splint. They object to being handled even after they have been in captivity a long time, probably because of the unpleasant warmth and dryness of the hand.

Cluster of eggs attached to a stone. (Two thirds natural size.) (Photographed by Dr. Miller.)

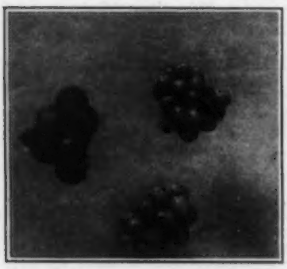
They appear to be very quick of hearing. Abbott, among other statements about the salamanders, mentions "their quickness of hearing." When undisturbed in their haunts, a whistle, clap of the hands, or a speaking voice sends them away. Abbott is of the impression that the salamanders give evidence of greater intelligence than the toads or frogs. His efforts to prove them possessed of cunning were not successful.

Reproductive Habits.

Baird writes of the genus *Plethodon*, "The eggs are deposited in packages or aggregations, in moist situations, under stones

and logs, not however, in the water; and the larvæ lose their branchiæ at a very early age."

Montgomery describes one finding of eggs: There were five eggs under a stone in July with an adult female curled about on guard. The eggs were large, enclosed in gelatinous envelopes, and curled about a large, nearly spherical yolk mass.



Eggs of dusky salamander (two clusters at the right), and red back (one cluster at the left.) (Natural size.) (Photographed by the author.)

Miss Sampson says, "*Plethodon* lays its eggs on land."

Ritter and Miller describe the eggs of *Aurodax lugubris* Hallow, a Californian salamander closely related to *Plethodon*. They are attached singly to the under surface of stones. In one instance a female with fifteen eggs was found under a platform in front of a barn, in dry earth next the foundation wall. The eggs are laid in July, are about 6 mm. in diamaeter and hatch in fifty days.

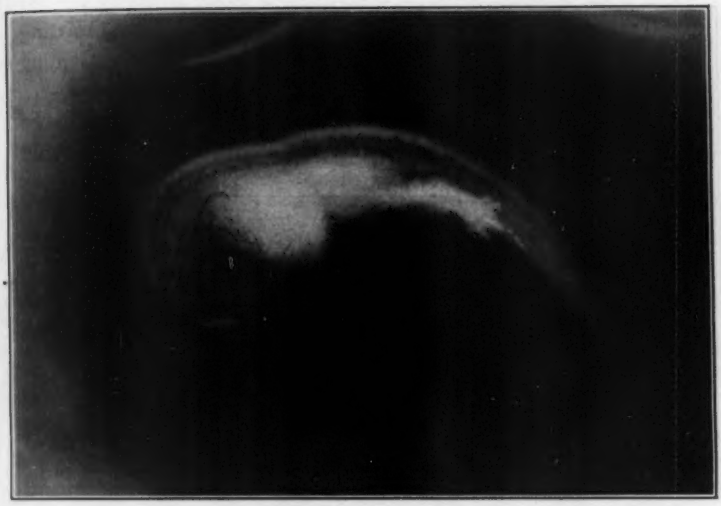
During the summer of 1910, eggs of the red-backed salamander were found first on July 5. From then until the latter part of August, eggs were found. They are in the ordinary habitats beneath stones and logs. The stones are always those deeply set into the ground in close contact with it, probably because there the atmosphere is moist.

The eggs, from five to nine in number, are in grape-like clusters attached to the under surface of stones or logs, unlike those of *Spelerpes* and *Autodax lugubris* Hallow, which are attached singly. One pedicel supports a cluster. This pedicel is tough, white, and seems made of separate threads closely stuck together; it resembles the tough, outer membrane covering each egg. There is no unifrom arrangement of eggs in the cluster.

The egg of the red-back is spherical, 5 mm. in diameter, has a prominent yolk and considerable transparent jelly. On the outside is a thin, tough envelope to which particles of earth and leaves readily adhere.

The embryo is coiled about the yolk. None were seen in

young enough stages to note the first appearance of color, but the red-brown of the dorsal stripe is visible at an early age.



Embryo red back, age unknown. (Enlarged about six times.) (Photographed by Dr. Miller.)

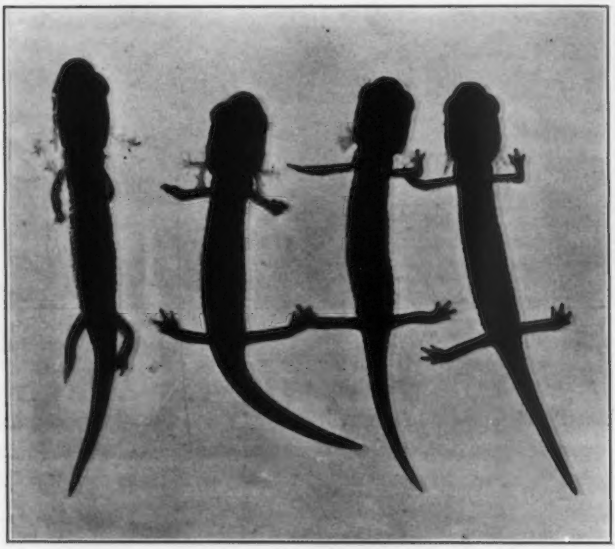
With each cluster of eggs, there was always one or two adults. Dissection showed that when there was one only, it was a female,

Embryo red-back taken from egg some days before time for hatching, showing the three pairs of gills. (Enlarged three times.) (Photographed by the author.)

when two, they were male and female. There is, accordingly, no evidence that the male takes any active part in the care of

the eggs in this species. The egg cluster is always so attached that one of the adults may coil its body about it. Sometimes a crevice beside a rock is utilized, the eggs, attached by the pedicel to a root or stone above, swinging loose. This brooding by the adults doubtless serves the double purpose of providing moisture and securing protection from insects.

Several attempts were made to transfer a parent with a cluster of eggs to the laboratory, that the development and hatching



From left to right: four embryos from the same cluster of eggs, one removed from the egg, one hatched about twelve hours, one about six hours and one about twenty-four hours. (Magnified three and one half times.) (Photographed by the author.)

might be observed. Damp moss was tried but it invariably moulded. A small jar kept moist with a wad of water-soaked cotton proved no better. Then, because the air of the laboratory seemed so disastrous, it was suggested opening the bottle, in which the eggs were brought from the field, upside down into an overturned sterile jar. Within the jar, the eggs were placed on a strip of filter paper that was so placed on a piece of glass

above a dish of water that it was constantly wet. No mould appeared, the moisture was constant, and the eggs developed.

An embryo just out of the egg is 2 cm. long; the gills are present but not quite so large as they are a day or two earlier. The head seems larger compared with the rest of the body, than in the adult stage. There is a large quantity of yolk visible which persists for several days.

In this cluster of eight eggs, five were allowed to hatch normally, three were opened for pictures. The hatching of the five covered a period of twenty-four hours. The actual process of hatching was not observed.

There is a small amount of jelly and membrane left; this was noted both in the laboratory and in the field where nests were under observation. Beneath one stone, the pedicel and gelatinous substance was observed after the eggs had hatched. It had turned dark and was tough and leathery.

The embryos just out are very active and show several characteristics of the species. Salamanders, less than a day old, avoid water, wriggle if annoyed and cling to the surface of the glass jar.

The gills shrivel rapidly; at the end of twenty-four hours, there are but mere stubs.

Summary.

In summing up the results of the observations reported above, the writer makes the following statements:

1. The red-backed salamander is found throughout all the eastern United States and Canada.
2. It lives beneath stones and logs, ranging from rather dry to wet places.
3. It is a small salamander, usually about three and one half inches in length.
4. It is almost entirely nocturnal. It is quick of motion and a climber; it cannot live in water.
5. The food consists mostly of live insects, larvæ, worms and spiders.
6. Snakes and frogs are probably its greatest enemies.
7. The protective devices are autotomy and the secreting of a viscous fluid from the tail.

8. The eggs are laid beneath stones in July and early August. One of the parents, usually the female, broods them. The young hatch with three pairs of gills which shrivel in about a day.

My acknowledgments are gratefully given to Dr. Clifton F. Hodge for suggesting the problem, for his active interest and help during the year, to Miss Louise Gulick for aid in collecting specimens and photographic work, to Mr. William T. M. Forbes for help in identifying insects, and to Dr. Newton Miller for his excellent and painstaking work in photography. I aslo wish to thank the many people, who by their interest, advice and criticism have aided in the work.

BIBLIOGRAPHY.

Abbott, C. C.

'84 Intelligence of Batrachians. Science, Vol. III., no. 50, Jan. 18, 1884, pp. 66–67.

Allen, Glover M.

'09 Notes on the Reptiles and Amphibians of Intervale, N. H. Proc. Boston Soc. Nat. Hist., Vol. XXIX., 1909, pp. 62–75.

Baird, Spencer

'69 Inconographic Encyclopedia, Vol.II., New York. 1869,

Boulenger, G. A.

'82 Catalogue of Batrachis Gradientia, London, 1882, p. 53.

Cope, E. D.

'89 Batrachia of North America, Bull. of U. S. Nat. Mus., no. 34, 1889.

DeKay, J. E.

'42 Nat. Hist. of N. Y. Zool. Part III., Reptiles and Amphibia, Albany, N. Y., 1842, pp. 75.

Ditmars, Raymond L.

'07 The Reptile Book, New York, 1907.

Esterly, C. O.

The Structure and Regeneration of the Poison Glands of Plethodon Oregonensis. Univ. Cal. Pub. Zool., Vol. I., no. 7, pp. 227–228.

Gadow, H.

'01 Cambridge Natural History. Vol. VIII., Amphibia and Reptiles. London, 1901.

Hodge, C. F.

'02 Nature Study and Life. Boston, 1902, pp. 296.

Holmes, S. J.

'06 Biology of the Frog. New York, 1906.

Hubbard, Marian E.

'03 Correlated Protective Devices in Some Californian Salamanders. Univ. Cal. Pub. Zool., Vol. VI., no. 4, pp. 157–170, Nov. 5, 1903.

Jordan, D. S.

'04 Manual of Vertebrates. Chicago, 1904.

Kingsley, J. S.
Standard Nat. Hist., Vol. III., p. 313.

Montgomery, T. H.
Peculiarities of the Terrestrial Larva of the Uredelian Batrachian, Plethodon cinereus (Green). Proc. Acad. Nat. Sci. Phil., Vol. VIII., pp. 503–508.

Ritter, W. E. and Miller, L.
'99 A Contribution to the Life History of Autodax Hallow, a Californian Salamander. Amer. Nat., Vol. XXXIII., no. 393, Sept., 1899, pp. 691–704.

Surface, H. A.
'06 The Serpents of Pennsylvania. Mo. Bull. of Div. of Zoöl. Penn. Dept. of Agriculture, Sept. 1, 1906, Harrisburg, Pa., pp. 208.

Towle, Elizabeth W.
On Muscle Regeneration in the Limbs of Plethodon. Biol. Bull., no. 2, pp. 289–305.

Whipple, Inez L.
'96 The Upsiloid Apparatus of Uredeles. Biol. Bull., no. 10, 1896, pp. 255–297.

ON THE DISTRIBUTION AND MODE OF OCCURRENCE IN THE UNITED STATES AND CANADA OF CLINOSTOMUM MARGINATUM, A TREMATODE PARASITIC IN FISH, FROGS AND BIRDS.

HENRY LESLIE OSBORN.

CONTENTS.

1. Geographical Distribution of *Clinostomum marginatum*..... 350
2. On the Cysts in the Black Bass..... 354
3. Behavior of Worms Liberated from Bass-cysts..... 357
4. On the Cysts in the Frog..... 360
5. On the Occurrence of the Worm in the Heron..... 362

1. GEOGRAPHICAL DISTRIBUTION OF *Clinostomum marginatum*.

The fluke referred to in this paper was first noticed in this country in 1856 by Joseph Leidy, in the intestine of the pike of the Delaware and in cysts attached to the gills of the sun-fish (*Eupomotis vulgaris*) near the city of Philadelphia. Leidy applied the name *Clinostomum gracile* to it. This generic name after many years of non-use has come into current usage since the late revision of the Trematodes which has received such impetus from the work of Looss ('00) who recognizes this name and Braun ('00) who in a revision of the genus in 1900 recognizes eight species of the genus *Clinostomum*, among them *C. marginatum*. In 1879 R. Ramsey Wright found cysts attached to the gills, branchiostegal membranes and pectoral fins of the yellow perch (*Perca flavescens*) at Toronto, Ontario, which he recognized as being identical with the *Clinostomum gracile* of Leidy and called *Distomum gracile*. His observation extended the geographical range of the species to the St. Lawrence River system. In the same paper Professor Wright reported the finding of *D. gracile* in the bittern (*Botaurus minor*) a fish-eating bird which was the first information as to the definitive host of the worm. Looss in 1885 published an account of the structure of a fluke which had been found encysted in the muscle of a silurid fish from Porto Rico. He gave

to this fluke the name *Distomum reticulatum* in allusion to the very peculiar excretory collecting apparatus. Subsequent writers, including Looss himself (Looss '99), have referred this worm to the species *Clinostomum marginatum*, and while there are certain points in which it differs from *C. marginatum* we may for the present so recognize it. This observation however takes the fluke a long way out of the region in which we otherwise know the animal and seriously embarrasses the attempt to deal with the geographical range of the species. In 1888 Leidy reported a fluke from the striped bass (*Roccus lineatus*) which he designated *Distomum galactosomum*. His account of the organization of this form touches on the form of the oral end of the body and the network of collecting vessels in the body-wall, two features of such peculiarity that we cannot doubt but that the subject of his study was none other than *Clinostomum marginatum*, which he had described in 1856 and evidently forgotten. His account located the worm in a marine host for the first time, unless Looss' silurid was marine.

In 1895 MacCallum ('99), of the University of Toronto, found a trematode encysted in the muscles of the frog which he regarded as identical with the ones which Wright had found in the fish and heron and referred to the *D. gracile* of Leidy. In his paper of 1899 MacCallum calls the worm *C. heterostomum*.

His description, while differing in some respects from my material, leaves no doubt but that our material is identical. I shall adopt the name *C. marginatum* in place of the name used by MacCallum following the lead of Braun ('00) in his revision of the group. He also found the same species in the throat of *Ardea herodias* at Danville, Ontario.

In 1898 Linton reported and figured this species under the name of *D. gracile* from the sun-fishes (*Eupomotis pallidus* and *Chænobryttus gulosus*) of Kansas City, Mo. This observation extended the range of the worm to a new river system, the Missisippi and gave us the most western point of its distribution. In 1900 specimens of this fluke were found by myself at Nebish, Mich., encysted in the muscular tissues of the small-mouthed black-bass (*Micropterus dolomieu*), also, though less frequently, in the muscles of the yellow perch (*Perca flavescens*). They were

also found later in the same year in the throat of the little blue heron (*Ardea herodias*). Nebish is a small camping place situated on the St. Mary's River, about twenty-five miles below the outlet of Lake Superior. A new locality was thus added to the distribution of the worm though one in the same river system as the Canadian ones.

In 1903 I received, through the kindness of Professor Linton, some pieces of the small-mouthed black bass infected with this parasite. The fish had been taken by the Rev. J. H. Young at Troy, Ohio, on the Miami River, a tributary of the Ohio, and part of the Mississippi system. Some of the worms were encysted in muscle, but others were located in the skin on the internal aspect of the branchiostegal membranes. Their location is shown in Fig. 1 of this article.

In the same year my attention was called by Dr. W. S. Nickerson, of the University of Minnesota, to certain flukes which he had found in frogs, and they were at once recognized as specimens of this species. At about the same time I began to notice them in the frogs of this region brought to the laboratory for use as biological material. The details of the occurrence of the fluke in this region is still under investigation in connection with a study of the parasites of the frogs of this vicinity, so that a fuller account of this part of the subject is reserved for a later occasion. It is important, however, to place on record the occurrence of the fluke in this region, as it extends the range of the parasite considerably beyond formerly recorded limits.

The latest appearance of this form in literature is the record of its recognition in the yellow perch of the Montreal markets by Stafford in 1904, where it is given Leidy's designation *Clinostomum gracile*.

The foregoing facts show that this species is very widely distributed in this country, having been recognized at Kansas City on the West and at St. Paul, Michigan, and as far east as Montreal. It has been seen as far north as St. Paul and as far south as Philadelphia and in Ohio in the center of this territory. The occurrence at Porto Rico is anomalous and cannot be considered until more information is forthcoming. In view of the wide distribution and large size of this species, it seems strange

that we are not in possession of a particle of information regarding the early stages of its life-history. The worm is fully developed when it is encountered in the fish or frog and all that is needed for its complete maturity is that the vitellaria shall become active, which they do as soon as the parasite reaches the heron. The details of structure of the worms of the bass have been determined both in total preparations and in serial sections. In the smallest individuals the entire organization is developed. It follows from this fact that there are three hosts involved in the life-history of this species of which the fish or frog is the second and the heron the final one, the first being unknown. The rôle of the fish or frog does not appear to be one of importance from the view point of the ontogeny, but seems merely to be for the purpose of getting the worm to the heron. It thus would appear that the primary host is not one which serves as food to the heron but does serve as food for the bass. This would fit the case of insects as well as any group of invertebrates.

In view of the fact that this parasite infects the edible portion of such important game and food fishes a knowledge of its life history is particularly desirable. While the parasite is fortunately one which is not injurious to the human system, at the same time its presence in the bass and perch unfit them for the table. It is clear that the worm is already widely distributed and is a menace to fish-culture, which at any time may become of great importance. It seems likely that if one were able to take up the problem of this life-history under favorable conditions for instance at some small body of water where the infected fish were abundant and to follow it up at various seasons the missing data could be obtained.

The data given in the foregoing paragraphs are summarized in the following table.

Date.	Observer.	Name Used.	Host.	Seat of Infection.	Locality.
1809	Rudolphi.	*Distomum marginatum.*			
1856	Leidy.	*Clinostomum gracile.*	*Esox.*	intestine.	Philadelphia.
1879	Wright.	*Distomum gracile.*	*Perca flavescens.*	branchiostegal membranes.	Toronto, Ont.
1879	Wright.	*Distomum heterostomum.*	*Botaurus minor.*	mouth.	Toronto, Ont.
1885	Looss	*Distomumi reticulatum.*	Silurid fish.	muscle encysted.	Porto Rico.
1888	Leidy.	*Distomum galactosomum.*	*Roccus lineatus*	——	Philadelphia.
1895	MacCallum.	*Distomum gracile.*	Frog.	muscle.	Toronto, Ont.
1897	MacCallum.	*Distomum heterostomum.*	*Ardea herodias.*	mouth.	Toronto, Ont.
1898	Linton	*Distomum gracile.*	*Eupomotis pallidus.*	pectoral fin.	Kansas City, Mo.
1898	Linton.	*Distomum gracile.*	*Chænobryttus gulosus*	roof of mouth.	Kansas City, Mo.
1901	Osborn.	*Clinostomum marginatum.*	*Micropterus dolomieu.*	muscle.	Nebish, Mich.
1901	Osborn.	*Clinostomum marginatum.*	*Perca flavescens.*	muscle.	Nebish, Mich.
1903	Osborn.	*Clinostomum marginatum.*	*Rana virescens.*	cœlom wall.	St. Paul, Mn.
1903	Young.	*Clinostomum marginatum.*	*Micropterus dolomieu.*	branchiostegal membranes.	Troy, Ohio.
1903	Young.	*Clinostomum marginatum.*	*Micropterus dolomieu.*	muscle.	
1904	Stafford.	*Clinostomum gracile.*	*Perca flavescens.*	gills.	Montreal, Ont.

2. Mode of Occurrence in the Black Bass and Perch, at Nebish, Mich.

The observations now to be described were made at Nebish in 1901 and repeated the following year. Nebish is a small settlement on the American side of the St. Mary's River about midway between Lake Superior and Lake Huron. At that time bass and perch were fairly numerous, beginning in the early part of September and the last of August. As they were not caught much earlier it was the local opinion that the fish migrate to the grounds at Nebish from elsewhere, and this is borne out by the fact that of late years, as a consequence of the work done in improving the channel of the river for navigation, the bass have ceased to come in the fall and the "fishing" is destroyed. I examined as many of the various kinds of fish and invertebrates as possible during my short stay at Nebish in hope

of getting some information which would lead up to the discovery of the first host. The only forms on which I recognized the parasites were the bass and the perch. Other fishes which were investigated with wholly negative results were the grass-pike, which is very often caught there, and the sun fish, *Eupomotis*—also very common. Various gasteropods and insects were examined but without any result. I have thus far not been able to obtain any clue to the source of infection of the fish.

The percentage of infected individuals found among the bass was very great. I do not happen to have any statistical data bearing on this point, but the parasite was found in nearly every bass submitted to examination, while in the perch it was much more rare. The cysts were very easily seen, being large, opaque and very creamy white, in marked contrast with the darker semi-translucent muscular tissue in which they lie imbedded. When the fish were skinned in preparing them for cooking the cysts walls were often torn open and the conspicuous worm seen moving on the surface of the meat. The cysts were found in all parts of the lateral muscles, deep and superficial, dorsal and ventral and headwards and tailwards. There was no evidence from their location bearing on the question of the mode of entry which had been adopted by the worms, but the observations of various others who have reported the worm from the gills, roof of mouth, branchiostegal membranes and pectoral fin would indicate that the worm enters the fish in the head region. If so it is difficult to imagine how they reach the places where we find the cysts if they are as large as we find them when they enter. On the other hand, if they enter as small and immature stages we should surely be able to find some evidence of it. Some of the worms should be less fully developed than others, or some of the waste products of the chemical processes involved in growth should be recognizable. So that we are unable to find a solution to this problem with the data at present available.

The number of cysts in single individuals varied greatly. The minimum number found was seven and the maximum number was more than one hundred. The appearance of one of the cysts in situ in the muscle is shown in Fig. 2. They are oval or spherical. They are smaller than those reported by Looss ('85) from

the fish which he examined, being 1.3–3 mm. long as compared with 3.5–4 mm. in length according to Looss.

The cysts occupy a space in the endomysium among the fibers of the muscle of the host. As we do not possess many detailed accounts of the structure of trematode cysts or their relation to the host tissues, an account of the facts found in this case may be of some interest. The following methods were employed. The cyst was carefully separated by teasing away the surrounding muscle fibers of a piece which had been fixed and hardened in suitable reagents. The cyst was then cut open and the enclosed worm removed, after which pieces of the wall were submitted to the action of various staining reagents and mounted for microscopical study. In other cases the muscle and enclosed cyst was sectioned serially. In such a series we have transverse sections of the wall and the surrounding muscle tissue, and at the ends of the series there are tangential sections which can be compared with the flat preparation just mentioned. Figs. 3 and 4 are low- and highpower views of such sections, and they show the structural factors involved. The muscle fibers are bent around the cyst as seen on the sides of the section; those in the center which seem to end abruptly at the surface of the cyst do not in reality do so, but are cut off as they run out of the plane of the section. The worm wholly fills the cavity of the cyst, the very small space which is seen in the figure being easily accounted for by the shrinkage of the worm. The structure of the cyst is seen in Fig. 4 to be merely a membrane produced by the condensation of fibrous tissue. There are the usual wavy fibers, thinning out as they reach the endomysium, and continuous with the fibers which fill in the spaces between the muscle fibers, and between the fibers the customary flattened connective tissue nuclei. The flat preparations and tangential sections show very clearly that the cyst is supplied with a capillary network derived from the vascular supply of the muscle, and these capillaries and their contained blood corpuscles can be recognized in the section of the wall as shown in Fig. 4. Looss states ('85) that his observations led him to conclude that the cyst is produced by the host and then lined by a second envelope produced by the worm itself. "Darunter befinded sich eine zweite Hulle, die, anscheinend ein erhärtetes Secret, von dem

Wurme um sich erzeugt wurde, und in dieser liegt eingerollt und zusammengeschlagen das Thier selbst." As to this inner lining found by Looss no traces of it are found in my material, and the cyst is wholly a product of activities on the part of the host tissues. The cyst may be looked upon as a defense made by the host against the parasite. It would be interesting to know the nature of the stimuli which have caused this reaction on the part of the supporting tissues of the muscle whether they are merely mechanical and acting in the form of pressure or whether they are chemical or both. The excessive formation of connective tissue and the growth of a definite and extensive capillary network have resulted from the presence of the worm. In the somewhat analogous case of the cavities produced in oak leaves by the sting of certain hymenoptera for the purpose of housing their larvæ, the normal growth of the leaf is turned aside sufficiently to produce cavities for the larva, walled with a material produced by the leaf from its own substance. In that case the stimulus would seem to be chemical. Whether it is so in this case cannot be determined until we are in possession of more facts connected with the introduction of the parasite to the tissue of the fish.

In the bass, as already stated, the worm entirely fills the cavity of the cyst. In order to determine its position within, several fixed and hardened cysts were carefully dissected out of their place in the muscle and the wall carefully removed under a dissecting microscope. The worms thus found bent and cramped within were macerated for an hour or less in tepid water till they became flexible and could be uncoiled as shown on Fig. 5. They were thus found to be bent twice, both times with the ventral surface outward, first on the level of the meeting of the first and second body thirds, and again on the level of the second and third body thirds. The first of the bends is in the median plane of the body and brings the dorsal surfaces in contact; the ventral sucker is thus exposed and serves to identify the surface posively. In making the second bend the body does not bend dorsally at once but first there is a twist which brings the dorsal side over the edges of the first and second thirds of the body and then the dorsal surface of the last body third laps over these

edges. The worms and cysts in the frog are very different from this. They will receive attention later.

3. Behavior of Worms Liberated from the Bass Cyst.

The living worms are easily liberated artificially by cutting into the cyst wall with a sharp instrument. As soon as a small opening has been made the worm begins to extrude through it almost as if it had been confined under some degree of pressure. It continues to make active movements of extension and contraction which soon result in freeing it from the cyst. It continues to move actively after gaining its freedom. In the course of nature the worm on emerging from the fish cyst would find itself in the stomach or intestine of a fish-eating bird; those which were removed artificially were received and kept in shallow vessels of fresh water where their activities could be watched. The surroundings in which they thus found themselves were quite unnatural and perhaps it was for this reason that they were so very active. The movements did not result in locomotion, though they produced constant changes in the form of the body, but the worm did not progress. Some trematodes adhere very forcibly by their suckers to any object with which they come in contact but there was no attempt made on the part of these to use the suckers for that purpose. Some of the various movements were so irregular and indefinite as to preclude the possibility of grouping them under any general head, but there were others which were definite and constant enough to be classed under either of two groups which I have called "poses" from the fact that in each case a series of movements took place culminating in a certain bodily form or attitude which was only momentarily maintained and after which the body relaxed and fell back to its ordinary resting posture. These two poses were seen often enough to justify the conviction that they are a constant feature of the behavior of the worm and so merit a detailed description. The two poses will be designated the "suctorial pose" and the "swimming pose." Sometimes the same pose is repeated several times in succession, at other times the two alternate irregularly.

The suctorial pose is represented in Fig. 6 as seen in the living animal and in Fig. 7, a view of a sagittal section of the anterior end of the body of an animal which happened to be caught in

this pose at the moment of fixation. The region of the body chiefly active in the production of this pose is the part anterior to the ventral sucker. Ordinarily this part of the body has the shape of an obliquely truncated cylinder. In assuming this pose the worm draws the end of the body back into the interior, the side walls being thicker and acting as the rim of a sucker projecting considerably beyond the level of the center. This inversion of the oral end of the body is brought about by the contraction of fibres of the parenchyma muscle system which run longitudinally in the body. Some of these fibres are shown in Fig. 7. Their contraction pulls the center of the oral end back and the margin is left projecting. If this action were to take place at a moment when the oral end was in contact with a soft surface, for example, such a surface as the mucous coat of the stomach or throat of the heron, then the soft substance of the host would be sucked into the cavity of this sucker and a powerful adhesion of the parasite to the host would result. A worm liberated naturally from the cyst would meet conditions which would furnish responses to this movement, so that instead of being merely a momentary pose it would be useful and so continued. On the other hand, in the case of the artificially liberated worm such stimuli being absent the worm returns to its customary form. The sucker thus formed is additional to the two usual trematode suckers. We may suppose that in as large a parasite as *Clinostomum* and one which lives in such an exposed place as the gullet where large masses of food are pressed against it additional adhesion would be needed to protect it against being dislodged by the pressure of the food as it is being swallowed. This striking movement had thus evidently a purposive character, as can be seen if we take into account the environment for which it has been developed.

The "swimming pose" is shown in Fig. 9. In this case the body is made broader and flatter than usual, the ventral surface becoming somewhat concave. The margins of the posterior part of the body are reduced to fine sharp lines like fins which do not extend into the front part of the animal but crossing it ventrally converge toward the ventral sucker. The line shown in the figure is not visible in any specimens of the worm after preservation and is only a momentary structure. The flattened

form of this pose too is only momentary, and no preserved specimens show it. In all cross sections of the worm the body is elliptical and has a thickness of nearly half its breadth.

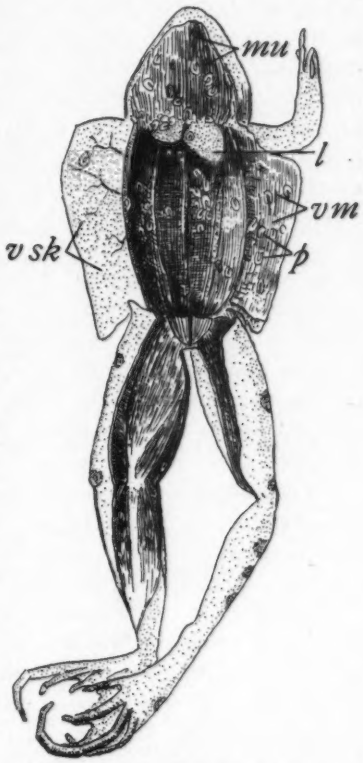
The transverse parenchyma muscles are the ones which are used in the swimming pose, the longitudinal muslces being at rest. Alternate contractions of the transverse and longitudinal muscles would throw the animal first into one, then into the other of these positions. The observation of certain leeches furnished the suggestion for calling this the swimming pose. In the leeches the margins of the body are thinned in this way and reduced to a thin lateral fin and the worms swim rapidly through the water. In the specimens of *Clinistomum* which were under observation no vibrations of the body were made and the pose was not turned to any account. It is possible that in nature this body form would be adapted to progression through the chyle and chyme of the bird and would be the means by which the worm reaches the throat toward which it is travelling from the stomach or intestine. It would be very interesting to experiment with these worms by removing them from cysts and placing them on surfaces as much like the avian mucous membranes as possible so as to ascertain whether these poses are as adaptive as they seem to be.

4. On the Cysts in the Frog.

The first mention of the frog as a host of *Clinostomum* is by MacCallum ('99), who reports it from the pectoral muscles of frogs found in Ontario. We have been finding it now for several years in the frogs caught in the vicinity of St. Paul which are used in our biological courses.[1] The mode of infection of the frog is different from that of the bass. The cysts, instead of being located in the muscular tissue, are seldom found there, but are in the peritoneal lining of the cœlom or in the lymph spaces between the skin and the muscles of various parts. Text-figure 1 is drawn from the most considerably infected specimen which I have met. The cysts are located in the cœlomic cavity;

[1] It is most probable that frogs and fishes in many parts of this country are infected by this parasite. Any information as to localities elsewhere where it has been noticed would be very gratefully received by the writer. A study of the relation of the worm to the frog is now in progress, and it is hoped that clues to the early history of the worm may be obtained through its connection with the frog.

especially in the region dorsal to the heart. They are found here most frequently. They are also found in the ventral wall of the cœlomic cavity, in the floor of the mouth between the muscles and the skin and even in the leg between the muscle and the skin. In no case have the cysts been found in the frogs of this region in the interior of muscular tissue surrounded by the fibers as they are so characteristically found in the fish. I have as yet not been able to find any reason for this difference in the location of the cysts with regard to the host, but the position of the worm within the cyst is considerably affected, as will be explained fully in a moment.



View of ventral surface after the removal of the cœlomic viscera, showing the numerous encysted flukes in place. *l*, lung; *mu*, muscles of floor of mouth; *p*, flukes in place; *v*, ventral muscles of cœlomic cavity; *v sk*, skin of ventral cœlomic wall. ×2/3 natural size.

The ratio of infection of the frog does not appear to be as high as it is in the fishes studied at Nebish. The percentage of frogs not infected is very high, indeed a large number can be gone through often without any cysts being found. The number of cysts in a single individual is usually not large; the frog figured is quite exceptional in that respect. Data for an exact statement on these points are not at present worked out.

The relation of the worm to the cyst and to the underlying muscle of the body wall is shown in Fig. 9. The muscle fibers

run along below the cyst, their course entirely uninfluenced by the presence of the cyst. A comparison of Fig. 9 with Fig. 2 shows the difference of habit in this respect at a glance. The preparation from which Fig. 9 was drawn shows five cysts in an area half an inch wide by a quarter of an inch across. It is removed from the right half of the body wall of the frog shown on page 361. The whole piece was stained and mounted in balsam and one of the cysts drawn in Fig. 9. The cyst is much larger than the worm and there are spaces within unoccupied by the worm. The worm in most cases is bent once, only the ventral surface being turned outward. In some cases the bend is not in the center, and then the longer end may be bent slightly as in the one shown in Fig. 9. The frog cysts show the presence of a rich network of capillaries spread out over their surface. These are derived from vessels which come from large and conspicuous vessels running in the space between the peritoneum and the muscle layer. The ordinary surface of the peritoneum does not possess these capillaries, which are evidently a growth developed as a result of the presence of the cysts.

The specimens of *Clinostomum* seen in the frogs are all virtually fully matured, having the full size of the heron specimens. Their inner organization too is identical in appearance with that of specimens from the fish, and excepting as to the vitellaria with that of the heron. There is thus no room for an hypothesis as to the frog being the first host and the medium by which the fish is reached, which hypothesis we might be tempted to frame, knowing that the frog is one of the foods of the bass, and other predaceous fishes. We are thus left to suppose a first host, possibly an invertebrate, followed by a second intermediate host, the fish which serves as a medium of transmission from the unknown first host to the heron. The case of a second host which serves to transmit a parasite from a first host where it develops to a final host where it matures and where sexual reproduction takes place has been recognized for a number of trematodes. A list containing 28 species is given by Braun (93, pp. 864–866), in which this species is not included. In some of these the frog or one of the fishes serves as the medium by which the final host is reached.

5. On the Occurrence of the Worm in the Heron.

A single specimen of the heron sent me by express from Nebish in October happened to be infected with this parasite and furnishes all that I have myself observed on this point. The bird had been shot and had been dead a day or two before it came into my possession. The worms were still alive and suitable for fixation and preservation for histological study. The worms were found in considerable numbers adhering to the wall of the throat by means of the anterior end used as a sucker in the manner referred to above. On being removed they were found to be mobile but no data as to their movements were recorded. The stomach and intestine of the heron were carefully examined to ascertain whether those organs were infected or not. No worms were found there. The stomach however contained the remains of the bodies of five fishes in various stages of digestion all of which were too decomposed for recognition with entire certainty but their elongate shape, their size and the fauna of the region made it virtually certain that they were sepcimens of the yellow perch. This observation furnishes the evidence of a connection between the fish and the final host. The liberation of the worms in the stomach and their migration of the worms in the stomach and their migration and adhesion to the wall of the throat is of course the first event of their life in the avian host followed by the production of eggs and their passage to the uterus where we find them in great numbers in the specimens taken from the heron. It is not exactly clear why the eggs are not passed directly from the body of the heron after the manner general in the flukes. Here however there is a large uterus into which the eggs pass and where they accumulate, for reasons as yet unknown. The eggs when they are expelled from the fluke must pass down the alimentary canal of the heron and fall with the feces in the water or less probably on the ground, where they make their way to the first host. The eggs in the uterus show no signs of development so that the ontogeny remains for the present a wholly unknown quantity.

Summary.

1. The first host and early life-history of *C. marginatum* are entirely unknown.

2. It passes a period of quiescence encysted in the muscle tissue of various predacious food fishes and in the lymph-spaces of frogs, in various localities ranging from Missouri through Ohio to Pennsylvania, and north as far as Minnesota, Michigan and Ontario, Canada.

3. It finally reaches an avian host which is some fish-eating bird, such as the bittern or the heron.

4. The cyst in the fish is a connective tissue structure produced from the endomysium of the host with a special vascular equipment.

5. The living worms on being artificially liberated from the cysts perform characteristic movements adapted to finding locations in the final host.

BIOLOGICAL LABORATORY,
HAMLINE UNIVERSITY,
SAINT PAUL, MINN.,
March 17, 1911.

LITERATURE.

Braun, M.
'93 Bronn's Klassen u. Ordnungen. Bd. IV., pp. 306–924. Trematoden.
Braun, M.
'00 Die Arten der Gattung Clinostomum Leidy. Zool. Jhrb. Syst., XIV.
Leidy, Jos.
'85 A Synopsis of the Entozoa, etc., no. 31. Proc. Ac. Nat. Sci. Phila., pp. 42–58.
'87 Notes on some Parasitic Worms, Proc. Ac. Nat. Sci. Phila., p. 24.
Linton, Edwin.
'98 Notes on the Trematode Parasites of Fishes. Proc. U. S. Nat. Mus., XX., pp. 597–548.
Looss, A.
'85 Beit. zur Trematoden, Distomum palliatum n. s. u. D. reticulatum n. s. Zeitf. w. Zool., XLI.
'99 Weit. Beitr. z. Kenntn. der Trematoden Fauna Aegyptens. Zool. Jahrb. Syst., XII., pp. 521–748.
MacCallum, W. G.
'99 On the species Clinostomum heterostomum. Am. Jour. Morphol., XV., pp. 697–710.
Osborn, H. L.
'01 Structure of Clinostomum. Science, XIII., p. 378.
Stafford, J.
'94 Trematoda from Canadian Fishes. Zool. Anzeig., XXVII., pp. 681–694.
Wright, R. R.
'79 Contrib. to Americ. Helminthology, No. 1. Proc. Canad. Inst., I.

Explanation of Plate I.

Fig. 1. View of the inner aspect of the right half of the lower jaw of a specimen of the small-mouthed black bass, from Ohio; showing the white cysts of *C. marginatum* at the bases of the branchiostegal rays, and black spots of an unidentified nature. Natural size.

Fig. 2. A cyst of *C. marginatum* in place among the muscle fibers of the bass from Nebish, Mich., from a glycerine preparation after teasing off part of the muscular tissue.

Fig. 3. View of a section passing through a cyst in place in the muscle tissue of the bass, from Nebish, Mich. Camera lucida, ×24 diameters.

Fig. 4. View from a section of a cyst in a plane transverse to the muscle tissue, showing in detail the histological structure of the wall of the cyst; from bass from Nebish, Mich. Camera lucida. ×340 diameters.

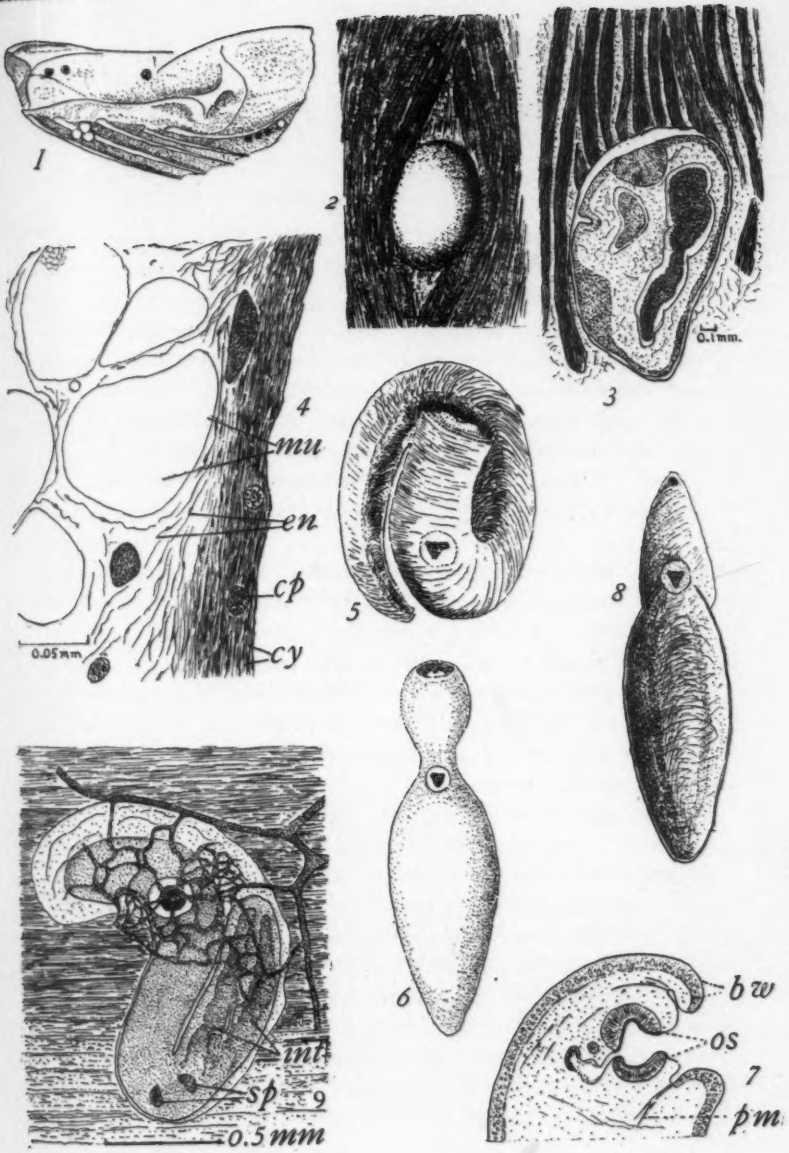
Fig. 5. Drawn from a specimen of *C. marginatum*, which had been removed from the cyst after fixation in alcohol and relaxed by slight maceration; from bass from Nebish, Mich.

Fig. 6. Sketch of living animal in "suctorial pose" as seen from ventral side; from bass, Nebish, Mich.

Fig. 7. From a sagittal section of anterior end of worm which died in "suctorial pose," from heron, Nebish, Mich. Camera lucida, × 38 diameters.

Fig. 8. View of ventral surface of a living animal in the "swimming pose," from bass, Nebish, Mich.

Fig. 9. View of a portion of the muscles of the ventral cœlomic wall of frog, showing a cyst and enclosed worm. From specimen after clearing and mounting in balsam. St. Paul, Minn. Camera lucida, × 35 diameters.



HENRY LESLIE OSBORN

THE SCALES OF FRESHWATER FISHES.

T. D. A. COCKERELL,

UNIVERSITY OF COLORADO.

Professor N. S. Shaler,[1] writing of his experiences as a student under Agassiz, said: "I acquired a considerable knowledge of the literature of ichthyology, becoming especially interested in the sytem of classification, then most imperfect. I tried to follow Agassiz's scheme of division into the order of ctenoids and ganoids, with the result that I found one of my species of side-swimmers had cycloid scales on one side, and ctenoid on the other. This not only shocked my sense of the value of classification in a way that permitted of no full recovery of my original respect for the process, but for a time shook my confidence in my master's knowledge."

I quote this, because the breakdown of Agassiz's original system of classification by scales affected not only Professor Shaler, but many others, to the detriment of lepidology. Dr. Jordan, in his great work, "A Guide to the Study of Fishes" (1905), devotes only two pages to the discussion of scales, with a couple of figures. In the descriptions of fishes published by various authors, much is made of the number and size of the scales, but little of their structure, and it rarely happens that an ichthyologist even takes the trouble to remove a scale from the fish he is studying, in order to determine its characters. These remarks apply especially to the teleosts—the ordinary fishes of modern times, and it is of course true that students of the "ganoids" and their allies, mostly fossil, have never neglected the scales. The ganoids have greatly thickened scales, usually rhombic in form, which are well preserved in the rocks; as Agassiz so well insisted, they afford a most important aid to the classification of these animals, particularly in the numerous cases in which the skeleton is poorly preserved. Recent researches, while destructive to certain theories which Agassiz considered im-

[1] *Atlantic Monthly*, February, 1909, p. 222.

portant, have only emphasized his doctrine that the scale is of great taxonomic value. I have paid little attention to the ganoids and their relatives, and will not attempt to discuss them, here, but may call attention to the important memoir by Goodrich[1] in which it is shown that the so-called ganoid scales can be divided into three very distinct groups, called the cosmoid, palæoniscoid ganoid and lepidosteoid ganoid. Mr. Goodrich, having distinctly differentiated these types, applies his work to the classification of some difficult forms, with striking results.

Teleostean scales, the ordinary imbricated readily removable scales of fishes, were classified by Agassiz as cycloid and ctenoid, and his names are still in current use. The cycloid scale is one in which the apical margin is what a botanist would call entire, that is without teeth or serrations. The ctenoid or comb-like scale has the margin dentate, serrate or spiny. It is now well-known that these are not fundamental divisions, some families having both ctenoid and cycloid types, and as Shaler has stated, it is possible for both kinds to exist on a single flat-fish. Nevertheless, the distinction is usually an important one, while the scales have innumerable other characters of value, not used in Agassiz's sytem of classification.

My own work with fish-scales had what might be called an accidental beginning. In the course of class-work, my students and I took occasion to examine the scales of the fresh-water fishes of Colorado. We soon perceived that they had excellent distinctive characters, and thereupon sought literature on the subject. Failing to find anything satisfactory, we wrote to Dr. D. S. Jordan, who promptly gave us the desired information, or rather, indicated the lack of it. Girard, in his report on the fishes of the Mexican Boundary Survey, had given numerous figures of the scales of fresh-water fishes, but had not discussed or classified them. About fifty years ago it appears that it was seriously intended to work up the lepidology of the American teleosts, but for some reason nothing came of it, beyond the publication of the figures mentioned. Later, Dr. Boulenger gave me access to the work of Fatio (1882), in which the scales of all the fishes of Switzerland are figured; also to the great work of

[1] *Proc. Zool. Soc. London*, 1907, pp. 751–774.

Sauvage on the fishes of Madagascar, containing numerous figures of scales of Acanthopterygian fishes. In other writings, here and there, are various figures of scales, usually with little discussion. The scales of *Gadus* (codfish and allies) have been beautifully figured and fully discussed by Dr. H. W. M. Tims (1905).[1]

Dr. Jordan, with great kindness, sent me a fine series of freshwater fishes from the collections of Stanford University, while Dr. Evermann was equally good in supplying numerous species from the Bureau of Fisheries. I thus obtained nearly all of the principal forms of North American Cyprinidæ or carp-like fishes. The following summer I visited the British Museum, and was indebted to Dr. Boulenger for the opportunity of investigating the scales of nearly all the principal old-world cyprinids, and a like series of African characinids. I also obtained through him many African fishes of other families. Quite recently I have received from Dr. Eigenmann, of the University of Indiana, an extremely fine series of South American characinids. With all these, and some others, it has been possible to test rather thoroughly the value of scale-characters, and the result has been to show that while they are not rarely deceptive, through convergence, they are on the whole of great taxonomic importance. As in most other taxonomic work, there are disturbing elements due to individual variability and differences of age, but while these are sure to lead to various minor errors, they will not much affect the broader results. The key to the origin of the sculpture of a teleostean scale is apparently to be found in that ancient type the bowfin of North America, *Amia calva*. Fig. 1 shows part of the base of a scale of this fish, which it will be observed consists of longitudinal strands or fibres, separable elements which fray out basally.[2] In the apical field these are directed toward a broad rough nuclear area. A close approximation to this is found in a very old type of teleosteans, the lady-fish, *Albula*. In this, however, appear also the beginnings of the radial lines, extending from the nucleus of the scale to the margin. As we go higher

[1] Dr. B. L. Chaudhuri informs me that Dr. John McClelland published an account of the scales of Indian Cyprinidæ in the appendix to his work on these fishes, in 1839. This I have not seen.

[2] See Smithsonian Misc. Coll., Vol. 56, No. 3, p. 2, 1910.

in the scale of fish-evolution, these radiating lines or *radii* often become very prominent, while the longitudinal strands usually become united above and below, forming circular fibers which we have designated *circuli*. The nomenclature of these structures was based on a normal highly-developed scale, in which the circuli deserved their name, and since then the term has been applied to the same elements wherever found, so that I have had to refer, rather illogically, to *longitudinal circuli*. Perhaps it would be better to call them fibrillæ.

On looking at a normal scale, in which the circuli are strictly circular, it would be natural to regard them as lines of growth, like those on a snail's shell. There are real lines of growth, however, and these do not necessarily coincide with or have anything to do with the circuli.

In the salmon and trout (*Salmo*) the scales are strictly cycloid, and have only circuli. Fig. 2 shows a scale of the bluefin, *Argyrosomus nigripinnis*, a member or the salmon family. This shows a marked deviation from the simple *Salmo* type, in that there are distinct laterobasal angles, the circuli of the apical field are less dense than those of the basal, and there are slight indications of apical radii. It is no very great step from this to the scale of *Moxostoma aureolum*, one of the suckers (Fig. 3), but it will be noted that in the sucker, as indeed in all members of the Catostomidæ so far examined, there are very distinct basal radii. In the typical suckers, *Catostomus*, the scale is oval, not unlike that of *Salmo* in shape, and there are radii all around. This is well shown in Fig. 4, *Pantosteus santa-anæ*, from California. The possession of basal radii separates the Catostomidæ from the great majority of American cyprinids, although a few genera of the latter family have them, notably *Chrosomus* (Fig. 5, *C. dakotensis*), the scale of which closely resembles that of *Pantosteus* in sculpture, though differing in shape. Among the old world cyprinids (carp family) basal radii are very common, and it is curious that the common European minnow (*Phoxinus phoxinus*) has scales of quite the same type as the American *Chrosomus*. There is reason to believe that the suckers are an ancient family, close to the old stem from which the carp family arose. They are nearly confined to North America, though spar-

ingly represented in eastern Asia. From this circumstance one might look for the earliest types of Cyprinidæ also in America, but the strong indications are that they are old-world forms, the modern American cyprinids, with a few possible exceptions, having arrived in comparatively recent times from Asia. Nevertheless, the carp family in this country must be only of comparatively, not actually, recent origin, since it has had time to develop innumerable species, and a considerable series of endemic genera. The distinctness of the American Cyprinid fauna has indeed been emphasized by the scale work, it being shown that the so-called *Leuciscus*, *Rutilus*, etc., of America are not congeneric with the European fishes bearing these names. It is probable, in fact, that all of the American Cyprinidæ are generically distinct from those of Asia and Europe.

Returning again to the bluefin scale, we can take a new point of departure in Fig. 6, the Asiatic cyprinid *Squaliobarbus curriculus*. Here the general form remains the same, except for a moderate elongation, the apical circuli become more widely spaced, the apical radii are evident, but there are no basal radii. A European scale of nearly the same type is that of *Leuciscus illyricus* (Fig. 7). In other cases, the scale may be more parallel-sided, with a broad truncate base, as is shown in the Asiatic *Labeo sladoni* (Fig. 8) and *Rhinogobio typus* (Fig. 9).

In order to illustrate the value of scale characters within a limited group, I give a series of figures of species usually referred to *Leuciscus*, the dace and its allies. *Leuciscus rutilus* (Fig. 10) and *L. friesii* (Fig. 11) are European; *L. hakuensis* (Fig. 12) and *L. jouyi* (Fig. 13) are Japanese. The remaining four are North American, and because of their very different scales, and other reasons, I have removed them to another genus, *Richardsonius* Girard. *R. orcutti* from California (Fig. 14) constitutes a distinct subgenus, the scale having basal radii. *R. pulchellus* (Fig. 15) is of the Rocky Mountains. *R. carletoni* (Fig. 16) is a distinct type from Maine, while *R. thermophilus* (Fig. 17) inhabits warm springs in Oregon. It will be observed that in the European forms the large scales have exceedingly fine circuli. In the American, the scales are very much smaller, and the circuli are very coarse and widely-spaced. This is in general a charac-

teristic distinction between New World and Old World Cyprinids, although some of the Old-World genera have widely-spaced circuli. The two Japanese species, as one might expect, are rather intermediate between the European and American. They probably should constitute a new subgenus or genus. *Leuciscus illyricus* (Fig. 7) has widely spaced apical circuli, and therein departs in the direction of the Japanese and American fishes.

The Characinidæ are a large family of freshwater fishes occurring in the Ethiopian and Neotropical regions, but not elsewhere, with the exception of a few which enter the Palæarctic in the Nile Valley and the Nearctic in southern North America. This curious distribution has naturally aroused interest among naturalists, and while it is probable that America was their original home, it is a question whether they reached Africa across what is now the Atlantic, or once inhabited the north and passed southward into the Ethiopian area. I should think the latter supposition more probable, but for the fact that as yet we have no trace of fossil characinids outside of their present area. The characinids belong to the same general group as the cyprinids, but are unquestionably more primitive, in spite of the fact that many of their members are exceedingly specialized in certain particulars. Among the African characinids is a group, the Alestini, having a very characteristic type of scale.[1] This, which I call the alestiform scale, is more or less hemispherical in outline, cycloid as to the margin, with a few very strong radii. It seems to be an old type, because we find it nearly repeated in certain of the relatively primitive families, as for instance the Phractolæmidæ (*Phractolæmus ansorgii*, Fig. 17*a*), a rare type from the Niger and Congo rivers. The *Phractolæmus* scale, however, is incipiently ctenoid. A different scale, yet of the general alestiform type, is found in *Pantodon buchholzi* (Fig. 18), another rare and isolated genus from the Niger and the Congo. On this fish the circuli have a peculiar and very characteristic bead-like appearance. Passing to the more specialized cyprinids, we find the alestiform scale not uncommon, and a good example from an Asiatic fish (*Barbus mahecola*, Fig. 19) is given. It will also be noted that *Leuciscus rutilus* of Europe (Fig. 10) has much the

[1] See Smiths. Misc. Coll., Vol. 56, No. 1, plate 1, figs. 4, 5, 6.

same sort of scales though lacking the characteristic lateral radii of the Alestini and the *Barbus*.

In some of the cyprinid alestiform scales the nuclear region is broken up into polygonal areas, but in the curious African Mormyridæ[1] there is a characteristic system of anastomosing radii, forming a network. This is unique, so far as material seen by me shows, except for the case of *Heterotis niloticus* (Fig. 20), a member of the ancient and now much reduced family Osteoglossidæ. In this fish, which inhabits tropical Africa north of the Equator, the network is extremely well developed.[2]

An extremely distinct and interesting type of scale is found in the tench of Europe, *Tinca vulgaris* (Fig. 21). This seems to be a relatively old type, formerly more abundant. Through the kindness of Dr. A. S. Woodward, of the British Museum, I was able to examine the miocene fossil species *Tinca furcata* of Agassiz, and the nominal but apparently synonymous *T. magna* of Winkler, and found the scales to be wholly characteristic of the genus. It is a singular thing that in North America there exists a fish called *Algansea tincella*,—named *tincella* by Valenciennes because of its resemblance to a tench,—which has scales of the same general type though not so extreme. Mr. Regan has described two other species of the genus *Algansea*, *A. affinis* and *A. stigmatura*, and he informs me that they have scales like those of *tincella*.

On the other hand, there are scales which so far as shape goes resemble those of *Tinca*, and yet offer marked differences in the details of the sculpture. Such is the scale of the Asiatic *Schizothorax biddulphii* of Gunther (Fig. 22). Among the American cyprinids, especially the smaller forms commonly known as minnows, there are many scales which at first sight look alike. In some cases, there is actually no definable difference, but often sufficient familiarity with the scales enables one to recognize them without difficulty. I illustrate a series of such forms, *Lavinia exilicauda* (Fig. 23), *Phenacobius mirabilis* (Fig. 24), *Notropis galacturus* (Fig. 25) and *Pimephales anuli* (Fig. 26); also a similar European scale, *Gobio fluviatilis* (Fig. 27). In all

[1] Smiths. Misc. Coll., Vol. 56, No. 3, p. 2, figs. 1, 2.

[2] Since this was written I have determined that all the Osteoglossidæ, and also all living Dipnoans (lung-fishes) have the radial network.

these scales there is much variation, which is at times confusing, although it usually does not affect the fundamental character of the pattern. Thus I described the scale of *Chondrostoma soëtta*, from Italy, as having few apical radii, distinguishing it thus from certain Spanish species. Noting later that this did not agree well with Fatio's figure, I asked Mr. Regan to look into the matter and he found that on the same fish immediately adjacent scales had many and few radii respectively. Consequently my diagnostic character proves of no value, but it will be noted that the variation affects the development rather than the character of the markings. Greater differences are noted when the scales are taken for different parts of the body, and for purposes of comparison I always take them from near the middle of the side, close to the lateral line. Fig. 28 and 29 show an extreme case, that of the curious little fish *Ericymba buccata*, from Indiana. Fig. 28 is a normal scale, and Fig. 29 is from the subdorsal region about 5 mm. in front of the dorsal fin. These two scales are from the same fish.

Occasionally very marked abnormalities occur. Thus on a specimen of *Ostebrama feæ* I found a greatly enlarged scale on the middle of the side, below the lateral line. It was about 8.5 mm. long, six times as long as the exposed parts of the normal scales, but not differing essentially in sculpture. The age of the fish is also often of importance. Fig. 30 shows a scale of *Gila robusta* from Arizona. It was taken from a young fish, and for some time I did not know that the adult scales of *Gila* have a characteristic basal lobe, as indeed was figured long ago by Girard in the report of the Mexican Boundary Survey.

There is an interesting case outstanding, in which the value of scale characters for the separation of very closely allied fishes has yet to be determined. From Stanford University I received a number of specimens of *Myloleucus symmetricus*, collected in different localities. These were sent principally because Dr. J. O. Snyder had already noticed the variability of this fish in other than scale characters, and it was suspected that it might include some separable forms. A study of the scales revealed four apparently distinct types. (1) Scale broad, small. Navarro River, Gualala River (California) and north to Drew Creek in

Oregon. (2) Scale broad, large. Napa River (California). (3) Scale oblong, more weakly sculptured. Ukiah Creek; Russian River (California). (4) Scale oblong, strongly sculptured. Conchilla Creek (California).

It appears from this that there is a broad-scaled form to the north coming south as far as Gualala River. From this it is only a few miles to Russian River, where the fish is oblong-scaled. Passing beyond, however, we find another broad-scaled variety in Napa River. It is suggested that the broad-scaled fishes, when in competition with the oblong-scaled, may get the better of them, and that the Napa River fish represents an invasion from the north away from the coast. All this, however, is at present based on far too little evidence, and is given here partly as an example of the kind of problem the scale-work brings up, and partly in the hope that someone will make an exhaustive study of the matter. Up to the present, I have not been able to get any further information, beyond what is given by Dr. Snyder in Bull. Bureau of Fisheries, XXVII, p. 175. In general, Dr. Snyder's results, based on other than scale characters, agree with mine, but he finds no difference between the fishes of Russian and Napa Rivers. No less than five specific names have been given to *M. symmetricus* as now understood; probably two or three of these will be available for the necessary segregates. While most fish scales fall readily into a systematic arrangement, we occasionally meet with one which differs greatly from any other known to us. Such is the scale of *Kneria cameronensis* Boulenger (Fig. 31), a small fish belonging to a peculiar family found only in tropical Africa. In this the radii run from one end of the scale to the other and the circuli are for the most part broken up into peculiar marks between them.

The study of fish-scales opens up a vast new field, the scales of most of the common fishes being still undescribed. Fish scales also may claim our attention for their beauty as microscopical objects. Their preparation involves no difficulty; they are best mounted dry between thin microscopical slides; or when small, they may be mounted on a slide under a cover glass. At first, I placed them in balsam, but this greatly obscures the markings, and is altogether undesirable.

For the means of obtaining the photographic figures (the work of Mr. T. C. Black) I am indebted to a grant from the American Association for the Advancement of Science. The drawings are by Miss Evelyn V. Moore. In this paper I have described and figured only scales of fresh-water species. At some future time, it may be possible to give a similar account of the marine families, and of several fresh-water groups now omitted.

EXPLANATION OF PLATE I.

FIG. 1. *Amia calva.* Plymouth, Indiana. Dr. Evermann.

FIG. 2. *Argyrosomus nigripinnis.* Dr. Graenicher.

FIG. 3. *Moxostoma aureolum.* Dr. Graenicher.

FIG. 4. *Pantosteus santa-anæ.* Santa Ana River, California. Stanford University.

FIG. 5. *Chrosomus dakotensis.* Valentine, Nebraska. Bureau of Fisheries.

FIG. 6. *Squaliobarbus curriculus.* Mountain stream near Kiu-Kiang (Styan). British Museum.

FIG. 7. *Leuciscus illyricus.* R. Tadro, Dalmatia (Dr. Werner). Brit. Museum.

FIG. 8. *Labeo sladoni.* Mandalay (F. Day). Brit. Museum.

1

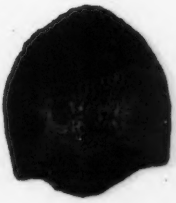
2

3

4

5

6



7

8

T. D. A. COCKERELL

EXPLANATION OF PLATE II.

FIG. 9. *Rhinogobio typus*. Kiu-Kiang (Styan). Brit. Museum.

FIG. 10. *Leuciscus rutilus*. Salisbury, England (Odgen Smith). Brit. Museum.

FIG. 11. *Leuciscus friesii* = *L. meidingeri*. Lake of Derkos, Constantinople. Brit. Museum.

FIG. 12. *Leuciscus hakuensis*. Yamada, Japan (R. Gordon Smith). Brit. Museum.

FIG. 13. *Leuciscus jouyi*. Sasuma, Japan (Anderson). Brit. Museum.

FIG. 14. *Richardsonius* (*Temeculina*) *orcutti*. Santa Ana River, California. Stanford University.

9

10

13

11

12

14

T. D. A. COCKERELL

EXPLANATION OF PLATE III.

FIG. 15. *Richardsonius* (*Tiogma*) *pulchellus*. Alamosa, Colorado. Stanford University.

FIG. 16. *Richardsonius* (*Cheonda*) *carletoni*. Cross Lake Thoroughfare, Maine. Bureau of Fisheries.

FIG. 17. *Richardsonius thermophilus*. Warm Spring, Harney Co., Oregon. Stanford University.

FIG. 17a. *Phractolaemus ansorgii*. Brit. Museum.

FIG. 18. *Pantodon buchholzi*. Brit. Museum.

FIG. 19. *Barbus mahecola*. S. Canara (F. Day). Brit. Museum.

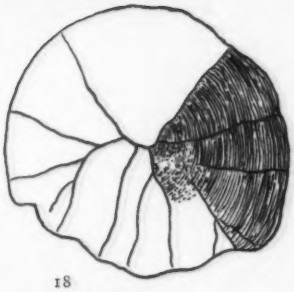
FIG. 20. *Heterotis niloticus*. Brit. Museum.

15

16

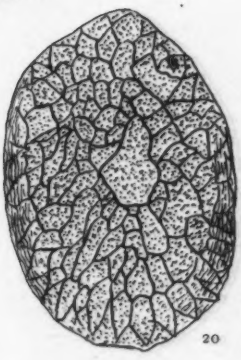
17

17a



18

19



20

T. D. A. COCKERELL

EXPLANATION OF PLATE IV.

FIG. 21. *Tinca vulgaris*. Constantinople. Brit. Museum.

FIG. 22. *Schizothorax biddulphii*. Lake Balyk-ky near Nijo. (St. Petersburg Mus.) Brit. Museum.

FIG. 23. *Lavinia exilicauda*. Coyote Creek, California. Stanford University.

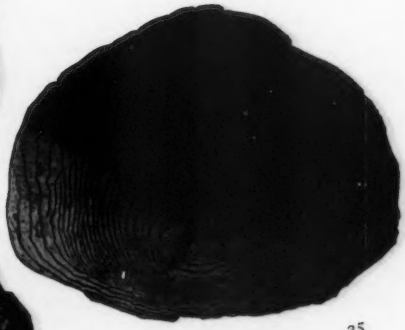
FIG. 24. *Phenacobius mirabilis*. Bureau of Fisheries.

FIG. 25. *Notropis galacturus*. Saltville, Virginia. Bureau of Fisheries.

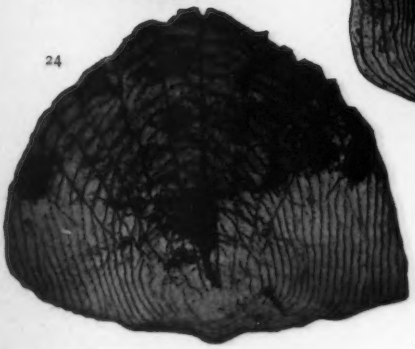
21

22

23



24



25

T. D. A. COCKERELL

EXPLANATION OF PLATE V.

FIG. 26. *Pimephales anuli*. Lunkasoos Lake, Maine. Bureau of Fisheries. (Note straight edge of skin across middle of scale.)

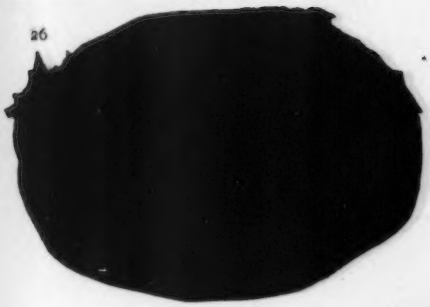
FIG. 27. *Gobio fluviatilis* (*G. vulgaris*). R. Neckar near Canstatt (Stuttgart coll.) Brit. Museum.

FIG. 28. *Ericymba buccata*. Wild Cat Creek, Indiana. Bureau of Fisheries. Figure reversed.

FIG. 29. *Ericymba buccata*. Wild Cat Creek, Indiana. From subdorsal region, in front of dorsal fin.

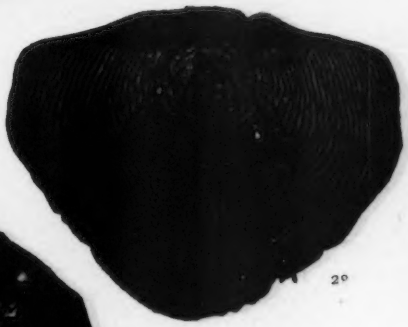
FIG. 30. *Gila robusta*. Tempe, Arizona. Stanford University.

FIG. 31. *Kneria cameronensis*. Brit. Museum.

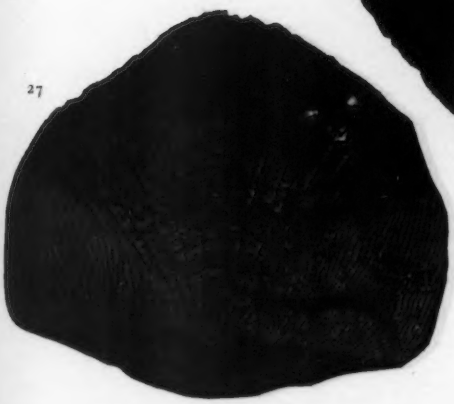


26

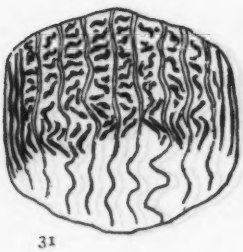
30



28



27



31

29

T. D. A. COCKERELL